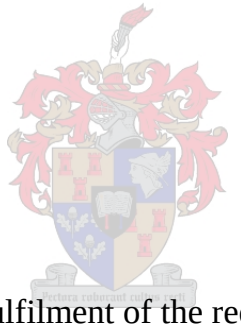
Trimen's False Tiger Moth, *Agoma trimenii* (Lepidoptera: Agaristidae): Biology and Potential Control Options

by

Courtney Anne Morris



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Summary

Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae), has developed pest status in vineyards in the Northern Cape and Limpopo (Groblersdal area) provinces of South Africa. Larvae feed on new vine growth and, if not detected early, the subsequent defoliation of vineyards can be severe, resulting in crop losses. Outbreaks are sporadic, and infestation levels vary. Little is known about the biology and behaviour of Trimen's false tiger moth, and no official monitoring methods or economic thresholds yet exist. Consequently, management and control options are lacking. Hence, attention has been drawn to the use of environment-friendly alternative pest control technologies. In this study, observational studies and visual scouting provided insight into the biology, seasonal development and behaviour of *A. trimenii*. The use of pheromone traps, live bait traps and light traps was tested as potential monitoring strategies of *A. trimenii*. The potential of different biocontrol agents, including entomopathogenic nematodes (EPN), entomopathogenic fungi (EPF), pathogenic bacteria and insect growth regulators were tested against *A. trimenii*, to be considered for later use in an integrated pest management (IPM) system.

The use of pheromone traps, light traps and visual scouting as potential monitoring strategies of *A. trimenii* was tested in the field. Various life stages of *A. trimenii* were identified, peak flight times were established, overlapping generations were determined, and the behavioural traits of all life stages were documented. Ultraviolet blue light traps proved to be the most promising potential monitoring strategy, with the prospect for an *A. trimenii* pheromone lure holding potential as an alternative monitoring strategy.

The susceptibility of larvae and pupae to EPNs of Steinernematidae and Heterorhabditidae and two commercially available EPF isolates, under laboratory conditions, were tested. The pathogenicity of two local species, *Steinernema yirgalemense* and *Heterorhabditis noenieputensis*, was screened against larvae and pupae of *A. trimenii*, using a concentration of 100 infective juveniles in 50 µl of water. After 48 h, 100% mortality of the larval stage was found. However, in the case of the pupae, no infection with EPNs was observed. The pathogenicity of two commercially available EPF isolates, *Metarhizium anisopliae* and *Beauveria bassiana*, was screened against larvae and pupae by means of a dipping test undertaken at a concentration of 0.2 ml/500 ml water and 0.5 g/500 ml water, respectively. At

15 days post treatment, 100% larval mortality was recorded. However, no mortality of the pupae was observed.

The susceptibility of larvae to three commercial products, Delegate®WG, Steward®150 EC and three different doses of DiPel®DF under laboratory conditions was examined. Semi-field trials were performed to test the potential of DiPel ® DF against larvae, applied at different water volumes (50g/1000L/ha and 42g/1200L/ha) and to compare spray coverage between top and bottom leaves on the vines. The residual activity of DiPel®DF when applied at different water volumes was investigated daily and compared between top and bottom leaves over a 7-day period. For the bioassay trials, Delegate®WG, Steward®150 EC and the recommended dose of DiPel®DF showed 100% larval mortality within 7 days, however Delegate®WG and the recommended dose of DiPel®DF proved to be the fastest acting products. The recommended dose of DiPel®DF (0.25g/500 ml distilled water) proved the most effective (in comparison to halved and doubled dosages) and showed 100% mortality after 5 days of application. Increasing the water volume of a spray application of DiPel®DF showed no significant increase in larval mortality for combined top and bottom leaves. Generally, bottom leaves displayed higher larval mortality compared to top leaves when treated with either water volume. A reduction in insecticidal activity for DiPel®DF applied at both water volumes was established between leaves picked 4 days after spraying and leaves picked 5 days after spraying, and no mortality was established after day 6. Bottom leaves displayed higher larval mortality throughout the 7-day period for both water volumes of DiPel®DF in comparison to top leaves, suggesting higher residual activity on bottom leaves of vines.

Future research should be aimed at increasing spray coverage and residual activity of DiPel®DF, as well as using all tested products within an IPM system. Results from the study can be used as recommendations for growers to monitor and control *A. trimenii* effectively and biologically, further contributing towards an IPM system for the moth.

Opsomming

Trimen se valstiermot, *Agoma trimenii* (Lepidoptera: Agaristidae), het pesstatus bereik in wingerde in die Noord-Kaap en Limpopo (Groblersdal area) provinsies van Suid-Afrika. Die larwes voed op nuwe wingerd groei wat kan lei tot grootskaalse blaarverlies en mislukte oeste, indien dit nie vroeg genoeg opgemerk word nie. Uitbrake is sporadies en die vlak van infestasie wissel. Min is bekend oor die biologie en gedrag van Trimen se valstiermot en geen offisiële metodes vir monitering of ekonomiese drempels bestaan al nie, wat beteken dat baie min opsies vir die beheer en bestuur van die mot bestaan. Daar word dus baie aandag gegee aan die gebruik van omgewingsvriendelike, alternatiewe pesbeheer tegnologie. Hierdie studie het, deur middel van waarnemingsstudies en visuele opnames, waardevolle insigte gelewer tot die biologie, seisoenale ontwikkeling en gedrag van *A. trimenii*. Die gebruik van feromoon lokvalle, lewendige aas lokvalle en lig lokvalle was getoets as potensiële monitering strategieë vir *A. trimenii*. Die potensiaal van verskillende biologiese beheermiddels was ook getoets teen *A. trimenii* vir moontlike gebruik in latere geïntegreerde pes bestuur (GPB) sisteme. Die middels wat getoets was, was entomopatogeniese nematodes (EPNs), entomopatogeniese swamme (EPSe), patogeniese bakterieë en insek groei reguleerders.

Die gebruik van feromoon lokvalle, lig lokvalle en visuele waarnemings was getoets in die veld as potensiële metodes vir die monitering van *A. trimenii*. Verskeie lewensfases van *A. trimenii* was geïdentifiseer, piek vlugtye was vasgestel, oorvleuelende generasies was waargeneem en die gedragskenmerke van elke lewensfase was gedokumenteer. Ultraviolet blou lig lokvalle was uiteindelik die mees belowende potensiële monitering strategie en ‘n *A. trimenii* lokmiddel het potensiaal getoon as ’n alternatiewe monitering strategie.

Die vatbaarheid van larwes en papies van *A. trimenii* tot EPNs van die Steinernematidae en Heterorhabditidae families, asook twee kommersieel beskikbare EPSe was getoets in laboratorium omstandighede. Die patogenisiteit van twee plaaslike EPN spesies, *Steinernema yirgalemense* en *Heterorhabditis noenieputensis*, was getoets op larwes en papies van *A. trimenii* teen ‘n konsentrasie van 100 infektiewe larwes in 50 µl water. Na 48 uur is 100% mortaliteit aangeteken by die larwe fase, maar geen infeksie van papies is gevind nie. Die patogenisiteit van twee kommersieel beskikbare EPS isolate, *Metarhizium anisopliae* en *Beauveria bassiana*, was getoets op larwes en papies teen ‘n konsentrasie van 0.2 ml/500 ml

water en 0.5 g/500 ml water, onderskeidelik. Teen 15 dae na behandeling was 100% mortaliteit by larwes aangeteken, maar geen by die papies.

Die vatbaarheid van larwes tot drie kommersiële produkte, Delegate®WG, Steward®150 EC en drie verskillende dosisse van DiPel®DF was ondersoek onder laboratorium omstandighede. Semi-veld proewe was uitgevoer om die potensiaal van DiPel ® DF te toets teen larwes by verskillende water volumes (50g/1000L/ha en 42g/1200L/ha), asook om die sproei dekking te vergelyk op die boonste en onderste blare van die wingerde. Die residuele aktiwiteit van DiPel®DF, wanneer dit aangewend is teen verskillende water volumes, was daagliks ondersoek en vergelyk tussen boonste en onderste blare, oor ‘n tydperk van 7 dae. Vir die biotoets proewe het Delegate®WG, Steward®150 EC en die aanbevole dosis van DiPel®DF 100% mortaliteit in larwes getoon na 7 dae. Delegate®WG en die aanbevole dosis van DiPel®DF was egter die produkte wat die vinnigste gewerk het. Die aanbevole dosis van DiPel®DF (0.25g/500 ml gedistilleerde water) was die mees effektiewe behandeling (in vergelyking met gehalveerde en verdubbelde dosisse) en het 100% mortaliteit veroorsaak na 5 dae. Toename in die water volume van ‘n sproei toediening van DiPel®DF het geen beduidende toename getoon in die mortaliteit van larwes in die boonste en onderste blare gesamentlik nie. Oor die algemeen het onderste blare hoër mortaliteit by larwes getoon in vergelyking met boonste blare wanneer dit behandel was met enige van die twee water volumes. ‘n Afname in insek aktiwiteit was aangeteken vir DiPel®DF aangewend by beide water volumes, tussen blare gepluk 4 dae na behandeling en blare gepluk 5 dae na behandeling. Geen mortaliteit was gevind na dag 6 nie. Onderste blare het hoër mortaliteit in larwes getoon oor die 7 dae tydperk vir beide water volumes van DiPel®DF, in vergelyking met boonste blare, wat ‘n moontlike aanduiding is van hoër residuele aktiwiteit op die onderste blare van wingerde.

Toekomstige navorsing moet fokus daarop om die sproei dekking en residuele aktiwiteit van DiPel®DF te verhoog, asook om die produkte wat getoets was te gebruik as deel van ‘n geïntegreerde pes bestuur (GPB) program. Resultate van die studie kan gebruik word as aanbevelings vir produsente om *A. trimenii* effektief en biologies te monitor en te beheer, wat verder sal bydra tot ‘n GPB sisteem vir die mot.

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Preface

This thesis is presented as a compilation of 5 chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture.

Chapter 1	**Literature review** Trimen's False Tiger Moth, *Agoma trimenii* (Lepidoptera: Agaristidae): Biology and Potential Biological Control Options
Chapter 2	**Research results** Seasonal Development, Biology and Behaviour of the Trimen's False Tiger Moth, *Agoma trimenii*
Chapter 3	**Research results** Laboratory bioassays on the susceptibility of the Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae), to entomopathogenic nematodes and fungi
Chapter 4	**Research results** Biological insecticides efficacy against Trimen's False Tiger Moth, *Agoma trimenii*.
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Chapter 1

Trimen's False Tiger Moth, *Agoma trimenii* (Lepidoptera: Agaristidae): Biology and Potential Biological Control Options

INTRODUCTION

The South African fruit export industry dates back over 130 years (De Beer *et al.*, 2003) and promises continuous growth, as the markets concerned are driven by global supply and demand. The industry is the greatest contributor, in terms of value, to South Africa's agricultural exports, as well as being a significant generator of foreign currency inflows, accounting for 45% of all agricultural exports (Ortmann, 2005; Uys, 2016).

The main export destinations include different European Union member states, with the leading export destination, the Netherlands, standing at 137 291 tons, followed by the United Kingdom, at 74 709 tons (South Africa. DAFF, 2015). South Africa's first crop estimate for the 2017/2018 table grape season was between 58.9 million and 63 million cartons, which is considered marginally higher than was the five-year average of approximately 57.9 million cartons for the 2012/2013 to 2016/2017 seasons (South African Table Grape Industry, 2017). Despite being the second greatest table grape-producing country in the southern hemisphere, following Chile, South Africa produces table grapes that are plagued by numerous insect pests, which tend to be absent from the countries to which they are exported. The above situation, in turn, can give rise to phytosanitary issues if the pest has the potential to follow the pathway of packed export fruit (De Villiers & Pringle, 2007; South Africa. DAFF, 2015).

The Trimen's false tiger moth, *Agoma trimenii* (Felder) (Lepidoptera: Agaristidae), which was first described in 1874 in subtropical Africa (De Prins & De Prins, 2012), has recently developed pest status in parts of South Africa, following on reports of moth infestations in vineyards in the summer rainfall areas (Pretorius *et al.*, 2012). *Agoma trimenii* is an indigenous Lepidoptera that is classified within the Noctuidae family, and the Agaristinae subfamily. However, members of the Agaristidae exhibit greater diversity in other parts of the world, where they are also known to attack vines. The painted vine moth, *Agarista agricola* (Donovan) (Beutenmüller) and the grapevine moth, *Phalaenoides glycinae* (Lewin), are both common

pests that attack vines in the arid regions of the United States of America and Australia (Australian Museum, 2010). However, these species have not, as yet, been reported from South Africa.

As the economic importance of *A. trimenii* in the grape-producing regions of South Africa increases, so does the necessity to conduct research into its biology and seasonal development, as scant research on this insect has, as yet, been done. Such basic information about the pest can be used to develop and implement integrated pest management (IPM) strategies against *A. trimenii*.

In the current review, all the available information on *A. trimenii* is summarised and critically discussed in the context of the information gained from field observations conducted in the Northern Cape, South Africa, during the 2016/2017 season. The study also includes the consideration of various aspects of the seasonal life cycle of *A. trimenii*, which were documented with a view to investigating, in future research, the potential biological control options available.

GEOGRAPHICAL RANGE

Both the introduction of *A. trimenii* to South African vineyards and its origin are uncertain, as no previous record of this moth species as a pest of any crop exists (Pretorius *et al.*, 2012). In addition to its broad dispersal within the Northern Cape and Limpopo provinces of South Africa, *A. trimenii* is known to occur in other African countries, including the Democratic Republic of Congo, Ethiopia, Gambia, Kenya, Malawi, Mozambique, Niger, Nigeria, Senegal, Sierra Leone, Somalia, Sudan, Togo, Uganda, Zambia, and Zimbabwe (Fig. 1.1) (De Prins & De Prins, 2012; Pretorius *et al*., 2012). No reports of occurrences of the *A. trimenii* beyond the African continent exist. Such restricted global distribution could present phytosanitary implications for table grape exports from South Africa. Coming to an understanding of *A. trimenii*'s association with its host plant, and parts thereof, was, therefore, deemed necessary, in terms of investigating the former's life and seasonal cycle, and how it relates to table grape production.

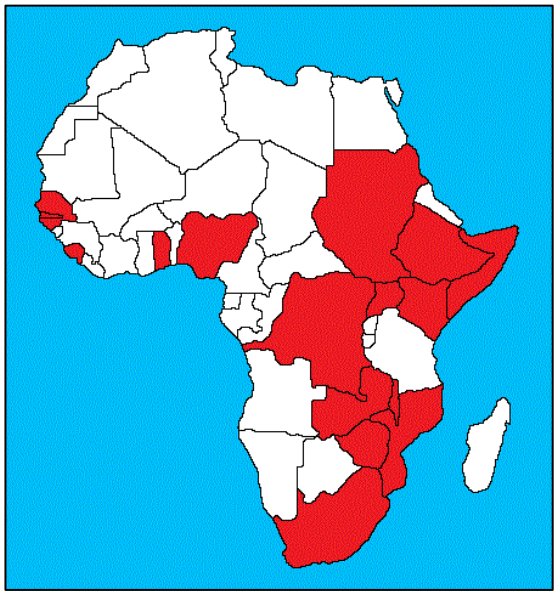

FIGURE 1.1

Geographical range of *Agoma trimenii* shown in red (De Prins & De Prins, 2012).

HOST RANGE

The feeding of the larvae of *A. trimenii* is described as being host-specific (Picker *et al.*, 2002). Known host plants are wild grapes (Vitaceae), belonging to the *Cissus* and *Rhoicissus* genera (Pretorius *et al*., 2012). Reports of damage to the leaves of *Cyphostemma cirrhosum* (Thunb.) (Vitaceae) have also been recorded (De Prins & De Prins, 2012). Within the Limpopo and Northern Cape provinces, damage to the leaves of table grapes, wine grapes and raisin grapes has been reported (Pretorius *et al.*, 2012).

MORPHOLOGY

Adults

Adults of *A. trimenii* are of medium size, with a wingspan of approximately 54 mm. A black-grey border outlines the black forewings, with there being a cream-coloured oval patch near the tip, and a triangular cream-coloured patch near the base. Hind wings are orange to yellow, with a black border. The bright orange hindwings are outlined by a black border (Fig. 1.2A).

The moth's orange-coloured abdomen (Fig. 1.2B) matches the hindwings, with a single longitudinal black stripe running down the centre of the abdomen (Pretorius *et al.,* 2012).

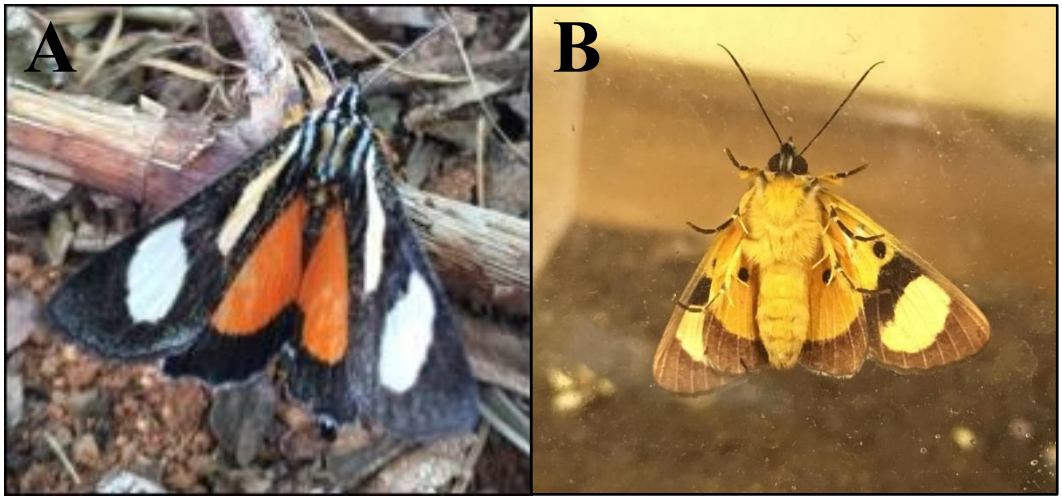

FIGURE 1.2

A: Top side of Trimen's false tiger moth, *Agoma trimenii*; B: underside of *Agoma trimenii*.

Eggs

Newly laid eggs are light green in colour, becoming cream-coloured, with irregular markings, as they mature (Fig. 1.3) (Pretorius *et al*., 2012). The eggs are almost impossible to see with the naked eye.

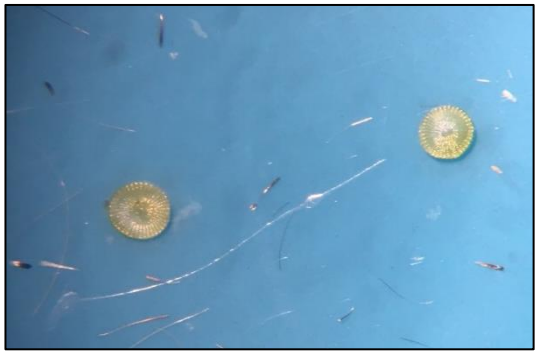

FIGURE 1.3

Eggs of *Agoma trimenii*.

Larvae

The first instar larvae are smaller than 0.5 mm. As the larvae develop and increase in size (± 3 mm), they display a cream-white colour. Older larvae are characterised by black and yellow markings that band their width (Fig. 1.4). An orange hump at the end of their bodies is a possible defence mechanism against predators. Later instars expel a green liquid droplet from the mouth when disturbed. Final instars are black in colour, measuring approximately 4 cm in length (Fig. 1.4).

FIGURE 1.4

Different larval stages of Agoma trimenii.

Pupae

When final instar larvae stop feeding, they migrate via a silken thread towards the soil, where they form prepupae before pupating in the soil as pupae that are surrounded by a hardened cuticle. The pupae are approximately 25 mm in length, with a dark brown-red colour (Fig. 1.5A). Holes in the soil, from which the larvae have burrowed, are visible, with a diameter of ± 2 cm and with a depth of ± 4 cm (Fig. 1.5B).

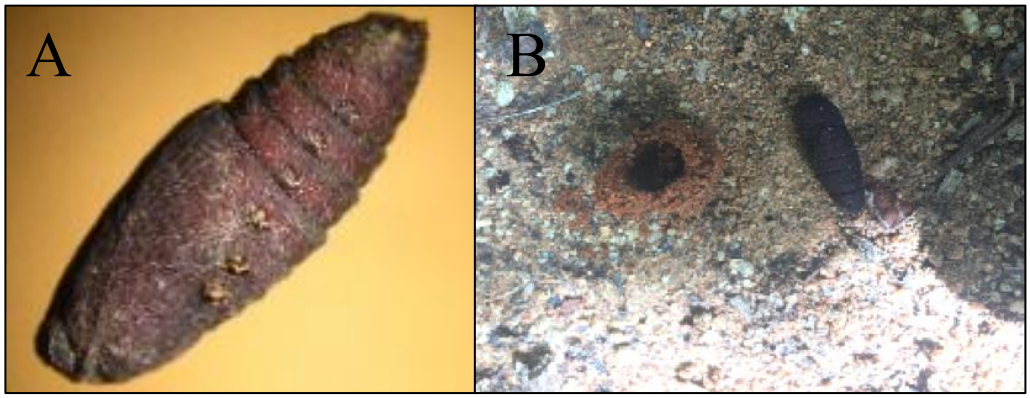

FIGURE 1.5

A: Pupa of *Agoma trimenii*; B: site of pupation in the soil.

LIFE AND SEASONAL CYCLE

Moths are visible in vineyards between October and March, predominantly during the harvesting of wine grapes during January (Pretorius *et al.*, 2012). Female adult moths deposit their eggs singly on the grape leaf surfaces, from which the eggs begin to hatch after approximately three days (Pretorius *et al*., 2012). The larvae feed on new shoots and leaves throughout the growing season, causing extensive damage to the vines concerned. Towards the end of March, when the growing season draws to a close, the larvae pupate and emerge as adult moths when the temperatures become favourable again.

CONTROL OPTIONS

To date, control measures entail the repeated application of chemical insecticides targeting the *A. trimenii* larval infestations. Such insecticides, which are currently undergoing registration, might threaten to induce the development of secondary pests that are currently under natural control (Pretorius *et al*., 2012). According to the survey conducted in 2011, infestation levels were so severe, in regions of the Groblersdal area, that the farmers concerned applied insecticides every 10 days (from October to March) to control larval infestations (Pretorius *et al*., 2012). Farmers were reported as rotating different insecticides to prevent resistance development. So-called ‘official registration trials’ were performed during the 2016/2017 season on two farms in the Northern Cape by Philagro South Africa Pty Ltd (a supplier of

chemical products to the agricultural sector in Southern Africa), in partnership with ExperiCo (a company offering scientific research and commercial services to the agriculture industry in Southern Africa). Both DiPel®DF (*Bacillus thuringiensis*) and the chemical Sumipleo™ (Pyridalyl) (in which the active ingredient is a dichloropropene derivative) were tested and achieved high success levels in the controlling of larval infestations (J.Y. de Waal, personal communication, 2017).

Despite the suppression of pest populations by means of insecticides, undesirable effects are often unavoidable (Blomefield, 2003). Insects that are genetically resistant to chemicals are usually selected, with nontarget organisms, including the natural enemies of the pest, being wiped out (Gullan & Cranston, 2014). Pesticides are then used to reduce the level of natural biological control agents (Wakgari & Giliomee, 2003). Consequently, pest resurgence can potentially exceed the levels found prior to insecticide treatment, if the natural predators recover at a slower rate than does the pest population (Gullan & Cranston, 2014). Secondary pest outbreaks are also potential setbacks that lead to insect species that were previously not regarded as pests being released from control, and potentially becoming major pests (Mgocheki, 2008). Concerns over human health (either in relation to the direct handling and consumption of the insecticides, or in relation to indirect exposure to environmental sources) and environmental impacts have encouraged the development and utilisation of alternate pest management programmes, products and technologies (Blomefield, 2003). Integrated pest management (IPM) strategies tend to address such concerns, and to accommodate new pest control techniques (Ahmad & Kamarudin, 2011). IPM can be regarded as a holistic enterprise that highlights the need to adopt a systems approach, creating synergies by means of integrating preventative methods, and by relying upon an array of solutions to provide the long-term sustainability of control methods (Barzman *et al.*, 2015). For an effective IPM system, a comprehensive knowledge of the pest insect's biology and natural enemies (in terms of the mortality levels that they exert, as well as their density and spatial interactions), in addition to the effects of other control techniques on the pest species, is required (Gullan & Cranston, 2014).

TRAPPING AND MONITORING

Trapping and monitoring for pest insects can, arguably, be regarded as being the first fundamental step to be taken in devising an effective IPM programme (Prasad & Prabhakar, 2012). The forecasting of the presence of insect pests is a crucial component of an IPM programme, as early warnings that are based on physical methods can provide lead time for the control of approaching pest attacks, thereby allowing for the minimisation of crop loss, the optimisation of pest control, and reduction in cultivation costs (Prasad & Prabhakar, 2012). Determining pest population density and distribution in the field is also beneficial to farmers, as they are thereby provided with a better understanding of pest activity in the field, thus enabling them to make cost-effective and environmentally sound decisions (Binns & Nyrop, 1992).

Pests are monitored by means of various monitoring tools, including pheromone, pitfall and light traps. Data captured from the traps serve numerous purposes, including forming part of ecological studies, in tracking pest migration and timing pest arrivals within the agroecosystem, as well as pesticide applications. They can also be used to predict later generations, based on the size of previous generations (Prasad & Prabhakar, 2012). As little is, as yet, known regarding the biology and seasonal development of *A. trimenii*, the monitoring of its presence, behaviour and population in the field by means of the use of visual scouting methods, and light and pheromone traps, should shed light on the many questions that are asked about this pest.

Visual scouting in monitoring systems of vines allows for the collection of valuable data, including early signs of larval infestation, the time and location at which leaf damage and infestation occurs, as well as infestation intensity. Scouting procedures include vine inspection at set time intervals and the classifying of each vine as infested or uninfested, by means of inspecting a predetermined number of leaves (De Villiers *et al*., 2006). Such procedures form part of a system that is being developed for the monitoring of arthropod pests of table grapes, with it having broad applicability to the monitoring of pest populations in all types of South African vineyards, supplying a foundation for the development of IPM as a control for *A. trimenii*.

The application of ultraviolet light traps has proven to be a promising method of surveying nocturnal moth populations, which exploits the attraction of such moths to artificial light (Jonason *et al*., 2014). The major advantage of light trapping is the immense number of species that can be recorded over a relatively short time period (New, 2004). To improve the likelihood of application for the purpose of population monitoring, environmental factors and their impacts on light trap efficacy require study (Steinbauer, 2003). Environmental factors, including such weather parameters as temperature, wind speed and rainfall, might alter catch size (Jonason *et al*., 2014). Awareness of contributing factors should increase the likelihood of selecting the right time for the setting of traps to best advantage, so as to minimise the 'non-productive' effort exerted in the deploying of light traps when no moths are likely to be trapped (Steinbauer, 2003). Light traps provide information on the presence or absence of a species, as well as on the mapping distribution and clarification of phenology, through enabling the determination of flight periods that can be integrated into the planning of control measures (New, 2004). As *A. trimenii* displays high nocturnal activity, the potential use of light traps will be tested in the field, as a possible monitoring option.

Pheromone traps are reliant upon a sex pheromone-based lure to attract specific insect species, which are then caught on a sticky base, or funnel (Witzgall *et al.*, 2010). The captured insects can accurately reflect whether an insect species is present, and when its seasonal flight period begins (McNeil, 1991). Successful applications of sex pheromones can be attributed to their use in detecting and monitoring pest populations (Witzgall *et al.*, 2010). Sex pheromones are species-specific and active in small quantities, with the majority being considered as non-toxic to both animals and humans (Joshi, 2006). When the information that is extrapolated from the traps is used predictively, such as in assessing the amount of damage that is caused by the following generation of larvae, thorough comprehension of the pest's biology, and of the effect of the weather and the particular crop stage, on development is required (Witzgall *et al.*, 2010). Such information can facilitate the appropriate timing of pesticide application, whether it be directed towards the most vulnerable life stage, or at the 'window periods' when the pesticide would be likely to be least effective in manipulating other pests or beneficial species (Witzgall *et al.*, 2010). Furthermore, thresholds can be determined for the timing of control strategies (such as the spraying of insecticides) or of decision-making as to whether remedial action should be taken (Knight & Light, 2005). Pest monitoring by farmers and landowners can be

further facilitated by supplying them with information that involves both the current and the historical records of trap catches, infestation rates, climatic data, and the geographical distribution of the target insects (Witzgall *et al.*, 2010). Several studies have demonstrated that lepidopteran species populations, like those of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera; Tortricidae), and of the codling moth, *Cydia pomonella* L. (Lepidoptera; Tortricidae), can be monitored using pheromone traps (Suckling & Burnip, 1993). Sticky plate traps can accurately reflect the highest trapping efficiency for monitoring in the case of the pine moth, *Thaumetopoea pityocampa* Denis & Schiffermuller (Lepidoptera: Noctuidae) (Jactel *et al*., 2006). The potential use of the pheromone belonging to the Australian grapevine moth, *Phalaenoides glycinae,* should be tested in the field against *A. trimenii*, as both species belong to the same family, *Agaristidae.* Additionally, live bait traps consisting of virgin female moths of *A. trimenii* will be tested in the field. Although the method of monitoring is an old practice, it still proves useful in monitoring situations where insects are present, as well as in the timing of flight peaks, as has been illustrated in numerous studies of different moth species, including cabbage looper moths, *Trichoplusia ni* (Lepidoptera: Noctuidae) and spruce budworms, *Choristoneura* (Lepidoptera: Tortricidae) (Miller & McDougall, 1973; Birch, 1977).

POTENTIAL BIOLOGICAL CONTROL OPTIONS

Although biological control involves the exerting of deliberate human intervention efforts to re-establish the balance of the abundance and distribution of species, by introducing, or intensifying, enemies in terms of their host specificity, it, unfortunately, does not completely eliminate pests. Therefore, biological control does not alleviate all the economic consequences of pests, but control systems are expected to diminish the abundance of a target pest to below the economic threshold levels (Gullan & Cranston, 2014). Potential biological control strategies of *A. trimenii* include the application of entomopathogenic nematodes (EPN), entomopathogenic fungi (EPF), insecticidal bacteria and derivatives like *Bacillus thuringiensis* (Bt) and *Saccharopolyspora spinosa*, and insect growth regulators (IGR). Such biological control agents exhibit many beneficial traits, including minimal risk to human health, as well as to beneficial and other nontarget insects, the absence of toxic residues on crops, and host specificity (Inglis *et al*., 2001). Testing the above-mentioned biological control options on *A. trimenni* is important, as no control options currently exist.

Entomopathogenic nematodes

Nematoda is an extremely diverse phylum, which occupies a variety of habitats, and which ranges from free-living nematodes feeding on soilborne bacteria and fungi, as well as on each other, to obligate plant and animal parasites (Machadoa *et al*., 2015). Entomopathogenic nematodes (EPNs), in the genera *Steinernema* and *Heterorhabditis*, are obligate parasites of insects, and are widely distributed in soils around the world (Hominick *et al*, 1997). The two genera have received the most attention throughout the years, as they possess many attributes of effective biological control agents (Lacey & Georgis, 2012; Malan & Hatting, 2015). The attributes include high virulence, and the ability to actively seek out hosts using chemoreceptors, making them promising alternatives to chemicals (Dowds & Peters, 2002). The entomopathogenic activity of both Steinernematidae and Heterorhabditidae families has been documented against a broad spectrum of insect pests in diverse habitats (Kumar *et al*., 2015).

Steinernematidae and Heterorhabditidae share similar life strategies, starting with a third-stage infective juvenile (IJ), or dauer juvenile (Griffin *et al.,* 2005). IJs can survive long periods in the soil while seeking a host, due to morphological and physiological adaptations (Ehlers, 2001). Once an IJ locates a host by using its chemoreceptors, it infects the host through such natural openings as the mouth, anus or spiracles, although heterorhabditid IJs can enter the host through the soft parts of the cuticle, by means of a dorsal tooth (Peters & Ehlers, 1997). Both nematode species have a mutual relationship with bacteria belonging to the Enterobacteriacaea family (Griffin *et al.,* 2005). Steinernematids are associated with bacteria from *Xenorhabdus*, whereas heterorhabditids are associated with *Photorhabdus* (Forst & Clarke, 2002). Once inside the haemocoel of the host, the IJs release their bacterial symbionts from their intestine. The bacteria then grow exponentially within the nutrient-rich haemolymph, while producing toxins and other metabolites that can result in the death of the host within 48 h after infection (Gaugler *et al*., 1997). The nematodes develop into feeding J3 juveniles, which feed upon the bacterial cells, as well as on the host tissue that is metabolised by the bacteria developing in the first generation and completing up to three generations, depending on the host's size (De Waal, 2008). Thereafter, the development of fourth-stage IJs occurs, with the IJs concerned then developing into adults of the first generation (Adams & Nguyen, 2002). The adults then

mate, and the females lay eggs that hatch and moult recurrently through four stages, with the fourth stage developing into adults. The process repeats itself, for as long as the host's cadaver provides sufficient food. Once the food resources from the host's cadaver are depleted, the offspring develop into special J3 juveniles, the IJs, which stop feeding and incorporate the symbiotic bacteria in the elementary canal before emerging from the cadaver, into the surrounding soil, in pursuit of a new host (Hazir *et al.*, 2003). *Steinernema* and *Heterorhabditis* employ different reproductive strategies. Heterorhabditids are hermaphroditic in their first generation (with the male and female species being closely related, with them deriving all their sexual traits from their dioecious ancestors) and amphitic in the following generations (with the gametes produced by males and females fusing and undergoing meiosis to form a zygote). Steinernematids reproduce by means of amphimixis in all generations, except one (Gaugler & Bilgrami, 2004; Griffin *et al*., 2005).

Currently, the only registered product (L9251) that is available on the market is sold by River Bioscience. The product name is Cryptonem, and the active ingredient is *Heterorhabditis bacteriophora* Poinar 1979. According to Hatting *et al*. (2018), the nematode is targeted towards codling and false codling moths, weevils and gnats. However, currently there are 14 locally occurring species of EPN reported from South Africa (Abate *et al*., 2018; Malan & Ferreira, 2017; Steyn *et al*., 2017), with *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams and *Heterorhabditis zealandica* Poinar already being in the process of mass cultivation (Ferreira *et al*., 2016). The potential of this nematode species should be investigated to improve the control of the different stages of *A. trimenii.*

Entomopathogenic fungi

Of the approximately 1.5 million species of globally distributed EPF, only 7.4% (110 000 species) have been described (Coombes, 2012). Of the above, almost 700 species, which are distributed over nine genera, are considered to be entomopathogens (Roy *et al*., 2010; Coombes, 2012). Almost 170 pest control products have been formulated based upon at least 12 different species of EPF (Roy *et al*., 2010). In South Africa, 10 fungi commercial products are available on the market, of which six are used against insects, and four are used against nematodes (Hatting *et al.*, 2018).

Members of the EPF species, *Beauveria bassiana* (Cordycipitaceae) and *Metarhizium anisopliae* (Clavicipitaceae), are soilborne microbial insect control agents that are used to control targeted pests, including larvae and pupae, at soil level (Goble *et al.*, 2011). EPF, in the form of mycoinsecticides, possess numerous desirable traits, including persistence in the environment for long periods of time, which means that they can provide long-term control (Cory & Ericsson, 2009). Despite their benefits, the major impact on pest populations may only occur once the economic thresholds have been exceeded (Goble *et al.*, 20011. Most EPF are soilborne, with all practising the same mode of infection (Castrillo *et al*., 2005; Goettel *et al*, 2005). Once fungal conidia encounter their host, they attach themselves to its cuticle by means of hydrophobic mechanisms, germinating when conditions are favourable, to form germ tubes (Inglis *et al.*, 2001). After the germ tubes have penetrated the cuticle, the mycelia grow in the host's haemocoel, producing external conidia, once the host dies (Inglis *et al*., 2001; Bidochka & Small, 2005). The strains of *Metarhizium* and *Beauveria* produce secondary metabolites, which have been shown to increase the rate at which insect hosts succumb to fungal infection (Coombes, 2012). Cyclic peptide toxins, known as destruxins, are produced by *Metarhizium* strains, with toxins like oosporein, beauvericin and bassianolide being produced by *Beauveria* strains (Goettel *et al.*, 1989; Castrillo *et al*., 2005). The strains are highly desirable for the development of bioinsecticides, as they display a 'double action' in killing their host, while strains that do not produce bioinsecticides only kill their host through hyphae growth within the host, which takes longer (Bidochka & Small, 2005). Despite both *B. bassiana* and *M. anisopliae* having already been successfully tested on many lepidopteran pests (Ranga Rao & Reddy, 1997; Ansarii & Butt, 2012; Chouvenc *et al*., 2012), no literature was found during the current research specifying that the fungi concerned have been tested on *A. trimenii*.

***Bacillus thuringiensis* subsp. *kurstaki* (Bt)**

Bacillus thuringiensis Berliner (Bt) (Bacillales: Bacillaceae) is regarded as the most extensively applied biological insecticide, especially against Lepidoptera (Broderick *et al.*, 2006). The species has also contributed to the first *Bacillus*-based products that became available in South Africa (Hatting *et al*., 2018). The five subspecies of commercialised Bt consist of *B. thuringiensis* subsp. *aizawai*, *B. thuringiensis* subsp. *israelensis*, *B. thuringiensis kurstaki*, *B. thuringiensis* subsp. *tenebrionis*, and *B. thuringiensis* subsp. *galleria*

(Chattopadhyay *et al*., 2017; Glare *et al*., 2012). Bt is characterised by numerous crucial attributes that favour its application in terms of a pest management programme, largely in terms of its non-toxic nature in relation to both plants and vertebrates, as well as its relatively specific action on target insect species (Lacey *et al*., 2015). As a gram-positive bacterium, it synthesises a proteinaceous crystalline inclusion within the sporangium during sporulation (Peralta & Palma, 2017). The inclusion is composed of crystal proteins, or δ-endotoxins, that display insecticidal activity towards the larval stages of lepidopteran, dipteran and coleopteran (Broderick *et al.,* 2006; Roh *et al.,* 2007). Once Bt is ingested by a susceptible insect, the δ-endotoxin is activated by alkaline conditions and enzymatic activity within the insect's gut (Schünemann *et al.,* 2014). If the activated endotoxin attaches to a receptor site, it paralyses and destroys the cells of the insect's gut wall, thereby allowing the gut contents to penetrate the insect's body cavity and bloodstream (Copping & Menn, 2000). Mortality of infected insects usually occurs within two to three days, as a result of the effects of septicaemia, or else it can occur directly (Waites *et al.,* 2009). Despite the few days' waiting period for the insect to die, it stops feeding soon after ingestion, and therefore stops harming the plants concerned (Copping & Menn, 2000).

Bt-based products, which are commercially available in many African countries, are registered, using the chemical plant protection pathway regulated by the respective Ministries of Agriculture, following the Food and Agriculture Organization's (FAO's) principles for their registration and use (Kabaluk *et al.,* 2010). The best known Bt-based insecticides are formulated from *B, thuringiensis* var. *kurstaki* isolates, which are specifically pathogenic to the larval stages of Lepidoptera, and which are known by the trade name, DiPel® DF (Aronson *et al*., 1986). DiPel® DF, which is a granule formulation that is mixed with water before its application, can be applied using any standard spraying equipment (Perez *et al*., 1995; Kay, 2007). No harmful residues remain on the crops after application, with no records of cross-resistance with other chemical insecticides having yet been documented, and no environmental damage having been caused, making DiPel® DF an ideal partner in terms of an integrated programme for the control of Lepidopteran pests (Kaaya, 1994). DiPel® DF has proven to be a successful biological control agent against two Lepidopteran pest species present in South Africa, namely the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), which is highly resistant to conventional insecticides, as well as the cabbage moth,

Mamestra brassicae (Linnaeus) (Lepidoptera: Noctuidae) (Tabashnik *et al*., 1990; Devetak *et al*., 2010).

The registration holder of DiPel® DF in South Africa is Valent Biosciences, with registration number L6441. As currently labelled, the product is effective against diamondback moth, semi-looper, army worm, pine emperor moth, African bollworm, leaf rollers, orange dog caterpillar, lucerne caterpillar, and lily borer (Hatting *et al.*, 2018). The product should, therefore, be tested against the different life stages of *A. trimenii*.

Spinetoram *Saccharopolyspora spinosa*

Spinetoram is an active constituent of biological substances known as spinosyns, originating from the soil actinomycete *Saccharopolyspora spinosa* (Shimokawatoko *et al*., 2012). The constituent is derived through the aerobic fermentation of a naturally occurring bacterium, *S. spinosa*, followed by ubsequent chemical modifications (Bacci *et al*., 2016). It was discovered by Dow AgroSciences LLC, in modification studies undertaken into fermenting the substances of *S. spinosa* (Shimokawatoko *et al*., 2012). Spinetoram, which is the active ingredient of the water- dispersible product Delegate® WG, is composed of two chemical compounds, spinetoram-J and –L, with each having a macrocyclic lactone structure (Watson *et al*., 2010). Delegate® WG boasts a broad insecticidal spectrum, especially against all the growth stages of lepidopteran pests, including codling moth, *Cydia pomonella*, light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), and oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) (Dripps *et al*., 2008; Magalhaes & Walgenbach, 2011; Sial *et al*., 2011). Its mode of action may be either directly through contact with the body surface, or indirectly through ingestion toxicity (Bacci *et al*., 2016). Spinetoram, which targets the nervous system of insects, does not interact with the known binding sites of other classes of insecticides, including pyrethoids, neonicitinoids, and carbamates (Besard *et al*., 2011). Delegate® WG is most effective when it is ingested, with it typically showing greater selectivity towards target pests and less activity against numerous beneficial predators, as well as against mammals and other aquatic and avian mammals (Angioni *et al*., 2011). Due to its unique mode of action, Delegate® WG could be an exceptional rotational product for use in an IPM programme against *A. trimenii*.

Insect growth regulators

Insect growth regulators (IGRs) can be classified as insecticides that cause a disruption to crucial physiological functions associated with the life cycle of insect development and metamorphosis (Sanchez-Bayo, 2012). IGRs are selective in their mode of action, and have potential to act only upon target species (Tunaz & Uygun, 2004). Compatibility with existing strategies within an IPM programme requires each component of the programme having a characteristic selectivity towards its specific target species (Tunaz & Uygun, 2004). Pesticide regulation has highlighted the introduction, or synthesis, of compounds (IGRs) that are specific to target hosts and that do not adversely influence beneficial and nontarget species. Consequently, direct approaches to exploring selective insecticides are currently utilised. Juvenile hormone analogues, such as methoprene and fenoxycarb, act as agonists of the hormonal system, preserving all insects in their larval stage by means of suppressing pupation, and thus preventing pupae from moulting into adults (Sanchez-Bayo, 2012). The highly active IGR, indoxacarb, is a broad-spectrum, pure insecticide that resides under the trade name Steward®. The insecticide is administered directly by means of contact through the body surface, or indirectly through ingestion (Wing *et al*., 2000). Indoxacarb's novel mode of action involves inhibiting sodium entry into the nerve cells, thus resulting in paralysis (inhibited feeding) and the death of the pest within three to five days (Dinter & Wiles, 2000). The foliar insecticide promises strong field activity against tortricid and heliothine lepidopteran pests (Wing *et al*., 2000). With its unique mode of action, and its minimal impact on beneficial parasitoid and predatory insects, it shows great potential as a component in IPM programmes (Nowak *et al*., 2001).

The above-mentioned biological control options can be classified as 'biorationals', which is an umbrella term that is used to characterise a variety of low environmental impact products that are biologically derived, or, if synthetic, structurally comparable and functionally identical to biologically occurring materials (Rea & White, 2013). The term has only recently been used to describe insecticides that are effective against the target pest, but which are less harmful to natural predators. At times, it has been used to describe naturally derived products, such as microbials, organic acids, plant extracts, pheromones, and other active ingredients derived from biological sources (Schuster & Stansly, 2005; Nath *et al*., 2017).

The application of numerous potential monitoring strategies and biological control options against *A. trimenii* is imperative for expanding insight into the pest to help control its growing population and the increasing damage that it poses against both the natural and human environment.

CONCLUSION

Urgent biological control of the pest is required to regulate the increasing population, to maintain healthy agroecosystems, and to ensure that the produce obtained for supply to commercial markets is of the highest standard possible. Testing the potential of monitoring options for *A. trimenii* populations can be a helpful way of accumulating information regarding the insect's biology and seasonal developmental stages. Such accumulation may result in the formulation of appropriate monitoring strategies that shed light on the timing and extent of control options against *A. trimenii*. Although the current control of the larval stages of *A. trimenii* has proven to be somewhat successful, no options have been tested against the diapausing pupal stages. Control of the stages concerned may be beneficial, as populations can be controlled before outbreaks occur and maximum leaf damage is caused by the different larval stages. All potential monitoring and control options can be examined for their effectivity and compatibility within the agroecosystem, thus contributing towards the development, and maintenance, of an integrated pest management system.

AIMS OF THE STUDY

The overall aim of the current study was to generate knowledge on the biology and seasonal occurrence of *A. trimenii* in South African vineyards, and to investigate the potential of various biological control options within an IPM system.

The objectives of the study were:

- to study and expand insight into the biology and seasonal development of *A. trimenii*
- to test the potential of pheromone traps, live bait traps and light traps as monitoring strategies used for *A. trimenii* populations in the field
- to screen EPNs and EPFs against larval and pupal stages in laboratory bioassays
- to screen *Bt* and IGR biorationals in laboratory bioassays

- to perform semi-field trials to test the potential of *Bt* when applied at different water volumes and assess spray coverage and residual activity of the *Bt*

As the dissertation appears in the form of individual journal publications, some repetition might occur between the different chapters. The format of the *South African Journal of Enology and Viticulture* was followed.

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Chapter 2

Seasonal Development, Biology and Behaviour of the Trimen's False Tiger Moth, *Agoma trimenii*

ABSTRACT

Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae), recently developed pest status in vineyards in the Northern Cape and Limpopo (Groblersdal area) provinces of South Africa. As little is known about the biology and behaviour of the moth, no official monitoring methods or economic thresholds relating to it, exist. Consequently, management and control options are lacking. In this study, the use of pheromone traps, light traps and visual scouting as potential monitoring strategies of *A. trimenii* was tested in the field. Various life stages of *A. trimenii* were identified, peak flight times were established, overlapping generations were determined, and the behavioural traits of all life stages were documented. Ultraviolet blue light traps proved to be the most promising potential monitoring strategy, with the prospect for an *A. trimenii* pheromone lure holding potential as an alternative monitoring strategy. Results from the study were used to develop recommendations for growers to monitor and control *A. trimenii* effectively and biologically, towards the development of an integrated pest management system for the pest.

Key words: *Agoma trimenii*, monitoring, grapevine, Trimen's false tiger moth, biology

INTRODUCTION

Table grapes are prone to attack by various arthropods, and damage may occur directly to the fruit, or indirectly by means of weakening the plant. Both adversely affect grapevine production. South Africa is a major contributor to the worldwide export of table grapes, having produced an estimate of between 58.9 million and 63 million cartons for the 2017/2018 season (Phaleng & Lubinga 2018; Uys 2016). In the aforesaid country, table grapes are infested by numerous insect pests, which are not present in the countries to which they are exported, such as the United Kingdom and various European countries, giving rise to phytosanitary concerns (De Villiers & Pringle, 2007). Additionally, markets impose legislative restrictions on the presence of insecticide residues on fruit, which further complicate the management of the pests, as well as of pests that are not of phytosanitary importance, but which cause economic damage. *Agoma trimenii* (Felder) (Lepidoptera: Agaristidae) is such an insect pest, with it currently affecting table grape production in South Africa (Pretorius *et al*. 2012).

Agoma trimenii is a noctuid moth, which, being indigenous to South Africa, is known to feed on several species of wild grape (Vitaceae) plants. Recently, *A. trimenii* has become a pest of cultivated grapes in the summer rainfall areas in South Africa. According to the first report on the moth, by Pretorius *et al.* (2012), the adults are known to be visible in vineyards between October and March, with the larvae feeding on new shoots and leaves, causing drastic defoliation, and subsequent crop losses. The larvae pupate in the soil mounds surrounding the vines, and overwinter until the next growing season. Although moth outbreaks are sporadic, population numbers have grown in certain table grape production areas in South Africa, and the development of control measures is imperative to prevent future crop losses. As the economic importance of *A. trimenii* in South Africa increases, so does the necessity to conduct research on its biology and seasonal development, as very little is known about it so far.

Many unanswered questions exist regarding the biology and life cycle of *A. trimenii*, highlighting the need for a monitoring system for the pest. Such parameters as peak flight times and foliar damage, as well as the duration, behaviour and location of each life stage, can be determined using monitoring strategies (Muirhead-Thompson 2012; Varela *et al*. 2010). Various methods of monitoring and surveying moths include the use of light traps, pheromone traps, and pitfall traps. The data captured from such traps serve numerous purposes, including

in terms of ecological studies, the tracking of pest migration, the timing of pest arrivals within the agroecosystem, and of pesticide applications, and the predicting of later generations, based on the size of previous generations (Prasad & Prabhakar 2012). Improved knowledge of *A. trimenii* should assist in decision-making and in the application of potential control options, towards developing and implementing an integrated pest management (IPM) strategy against the pest.

The aim of the current study was to gain enhanced understanding of the biology and behaviour of *A. trimenii*, by means of testing various trapping and monitoring strategies under field conditions. The above included observational studies and visual scouting; morphological observations of the different life stages; behaviour and damage; light traps; and pheromone and live bait traps. The study reports on the first of such observations being done on *A. trimenii* in South Africa, presenting a base to assist in future research, and in the evaluating and identifying of sample populations, research methods and potential problems with *A. trimenii* in grapevine.

MATERIALS AND METHODS

Field sites

The study was carried out on two commercial table grape farms in the Northern Cape province of South Africa, with high infestation levels of *A. trimenii*. Observational studies and visual scouting were conducted during the growing season and the harvest time of the table grapes, over a seven-month monitoring period, from October 2017 to May 2018 (week 41 to week 19), comprising a 33-week period. During the time, potential monitoring methods were also tested. Farm A (28°39′52.4″S 21°07′52.8″E) comprised a netted block of the table grape varietal, Thompson Seedless. The vines followed a double-gable trellising system, with plant spacing of 1.8 m, and row width of 3.3 m. Five rows of six vines were demarcated for use as the trial site. Five vines between two trellising posts of each of the six rows were used for monitoring. Farm B (28°67′84.4″S 20°39′59.9″E) was comprised a netted block of the table grape varietal, Sugraone. Similar to the situation on Farm A, the vines followed a double-gable trellising system. Two rows of 15 vines were demarcated for use at the field site. The demarcated areas at both of the farms were treated with neither pesticides nor insecticides for the duration of the study.

The temperature and relative humidity at both field sites was measured using Tinytag data loggers (Gemini Data Loggers Ltd, South Africa) and iButtons® (Maxim Integrated Products, United States). At Farm A, two iButtons® were placed in the vine canopy, on the first and fifth vine of the third row of the demarcated area. Another two iButtons® were placed in the soil mounds surrounding the first and fifth vine of the third row. A Tinytag data logger was hung in the vine canopy on the third vine of the third row. At Farm B, a Tinytag data logger was hung in the vine canopy on the seventh vine of the first row; however, the data logger was lost in the field, meaning that no data could be retrieved therefrom. One iButton® was placed in the vine canopy, on the eighth vine in the first row of the demarcated area. The iButtons® were programmed to record temperature at intervals of 2 h from December to May 2017/2018, and Tinytag data loggers were programmed to record temperature and humidity at 12 h intervals from November to May 2017/2018.

Observational studies and visual scouting

Presence/absence of A. trimenii moths and larvae

Observational studies and visual scouting were carried out in the demarcated field site on Farms A and B, during the monitoring period (weeks 41–19). Visual scouting for the presence of *A. trimenii* pupae, larvae and moths was recorded over the 33-week period on Farm A. In October, about one month after bud break, scouting began, by observing and noting the presence/absence of adult moths flying both in and around the study site. After shoot and leaf emergence, the inflorescences of the vine became visible, with the inspection continuing throughout the flowering and berry set, until the harvesting of the ripe bunches (weeks 41–49). One bunch per vine was examined for the presence of *A. trimenii* moths and larvae, as well as for any feeding damage. The presence/absence of larval infestations within the vine canopies was recorded throughout the growing and harvesting period. The pupae were scouted during the harvesting period by digging a radius of ± 80 cm in the soil around the first and last vine of each experimental row (10 vines), with their presence/absence being recorded.

Counts of adult moths and larvae

During the 33-week monitoring period, visual scouting was carried out over 7- to 10-day periods each month, so as to obtain counts of adult moths and larvae within the demarcated

field site. Scouting was conducted by following the generic sampling system for the monitoring of arthropod pests on vines, as outlined in De Villiers & Pringle (2008). The distal 15 cm of one shoot of every fifth vine per row was examined for the infestation and damage caused by *A. trimenii* larvae. Both the underside and the topside of the leaves were inspected, and the number of larvae were recorded by date. The adult moth counts were obtained and recorded by date, by means of counting the number of moths (flying or stationery) within, or around, the demarcated field site.

Description of life stages

The pupae of *A. trimenii*, after having been collected from Farm A in late May 2017 (n = 30) and in late June 2018 (n = 50), were transported to the quarantine laboratory at the Department of Agriculture, Forestry and Fisheries (DAFF) in the Western Cape. The pupae collected in May 2017 were stored under a thin layer of dampened soil in two Perspex containers (30 cm × 40 cm), at 26ºC, 12:12 L: D and 70 % humidity. Small cylindrical plastic containers, fitted with rolled cloth soaked in sugar water, were placed in each container as food sources for the moths that had emerged under such laboratory conditions. The pupae were observed every 2 to 3 days, for a period of 35 days. The pupae collected in June 2018 were measured, using a ZEISS stereomicroscope fitted with a camera, and ZEN microscope computer software. Various *A. trimenii* larval instars of different sizes (n = 10), collected at Farm A on 4 January 2018, were taken to the laboratory, where the width of the head capsule of each larva was measured using a stereomicroscope. The moths were observed in the Perspex containers from their time of emergence from the pupae until death, and their characteristic markings and colouration were described. The body size of the male and female moths was compared.

Behaviour and damage

The behaviour of the larval and the adult moth stages of *A. trimenii* was observed within the demarcated areas of both field sites, throughout the monitoring period (weeks 41–19). The larval damage done to leaves was observed, by inspecting the top and the bottom leaves of the vines, including the young shoots and leaves, and recorded within the demarcated areas of both the field sites concerned.

Light traps

During the 2016/2017 season (from mid-November 2016 to late December 2017), a light trap emitting blue ultraviolet (UV) light (Fig. 1.1A) was tested at Farm A to assess its potential as a monitoring option for *A. trimenii* moths. The light trap was placed at the perimeter of the block, and the moths that were attracted to the blue light, and which were caught during the night, were recorded, in terms of number, on a daily basis.

During the 2017 season (6–27 November), an alternative light trap (Fig. 1B) was made by hanging a solar-powered white LED (light-emmiting diode) light in the vine canopy (± 2 m high), and placing white sticky pads in close proximity to the emitted light, so that light was reflected from the hanging LED light onto the white sticky pads. The solar light trap was placed on the eighth vine in the second row at the Farm B study site. The light was charged during the day, with it being switched on just before sunset, so that it would last another 6 to 8 h. The light trap was placed in the field at the beginning of November 2017. The sticky pads were checked each morning, for 3 weeks, for the presence of adult moths, so that they could be replaced, if necessary.

FIGURE 2.1

A: Ultraviolet blue light trap used to trap *Agoma trimenii* moths during the 2016 season; B: the solar-powered LED light used during the 2017 season.

Pheromone and live bait traps

The semiochemicals of the grapevine moth, *Phalaenoides glycinae* (Lepidoptera: Agaristidae), were tested as a pheromone attractant for *A. trimenii*. The components of the pheromones of *A. trimenii* are unknown, and, since *A. trimenii* and *P. glycinae* are part of the Agaristidae family (de Prins & de Prins, 2012; Pretorius *et al.* 2012), the potential for them sharing the same or similar semiochemicals was considered and tested. Two yellow delta traps, containing a white sticky pad and fitted with pheromone rubber capsules containing the synthesised grapevine moth lure, were used (Fig 2.2A). The grapevine moth lure is a 50:50 combination of 2-phenylethanol and phenethyl acetate (The Pherobase [n.d.]), manufactured by Chempak (Pty) Ltd in Paarl, South Africa. The pheromone traps were placed in the demarcated field site on Farm A in early November 2017, at the height of the main cordon of the fifth vine in the first and fifth row, closer than before to the middle of the block. The openings of the traps were positioned parallel to the direction of the row, so as to avoid any drift from chemicals being sprayed in other parts of the vineyard. The traps were checked every day, for three weeks (6 November – 28 November 2017), for the presence of moths, with the sticky pads being replaced, if necessary.

Caged female virgin moths were also tested as lures to attract and trap adult moths. The female pupae were separated from the male pupae by distinct characteristic markings on the abdomen (Van den Berg, pers. comm., 2017). Once they were separated, the pupae were left alone until the adult female moths emerged. Two yellow delta traps containing a white sticky pad were fitted with a small cage, with each containing one female moth (Fig. 2.2B). The first trap was placed in the demarcated field site on Farm A, at the height of the main cordon of the third vine in the third row. The second trap was placed in the demarcated field site on Farm B, on the twelfth vine of the first row. The openings of the traps were positioned parallel to the direction of the row, so as to avoid any contaminating drift from the chemicals being sprayed. The traps were placed in the field during December 2017, when the temperatures were, on average, ± 35°C. The females were replaced if they were found dead inside the traps, and each trap was checked every day for a week (2 December – 9 December 2017), for any moths that had been captured on the sticky pads. The sticky pads were replaced whenever it became necessary to do so.

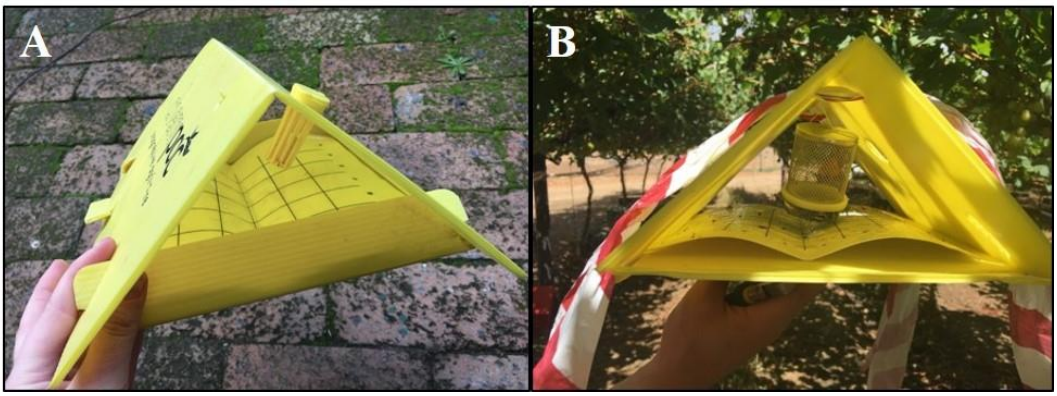

FIGURE 2.2

A: Pheromone trap containing grapevine moth lure; B: live bait trap containing a virgin female *Agoma trimenii* moth.

RESULTS

Observational studies and visual scouting

Agoma trimenii has several generations per year, with the life cycle consisting of the egg, numerous larval instars, the pupa and the adult (Fig. 2.3). The first moth emergence was documented in mid-October 2017, and the presence of moths lessened in the field in mid-May 2018.

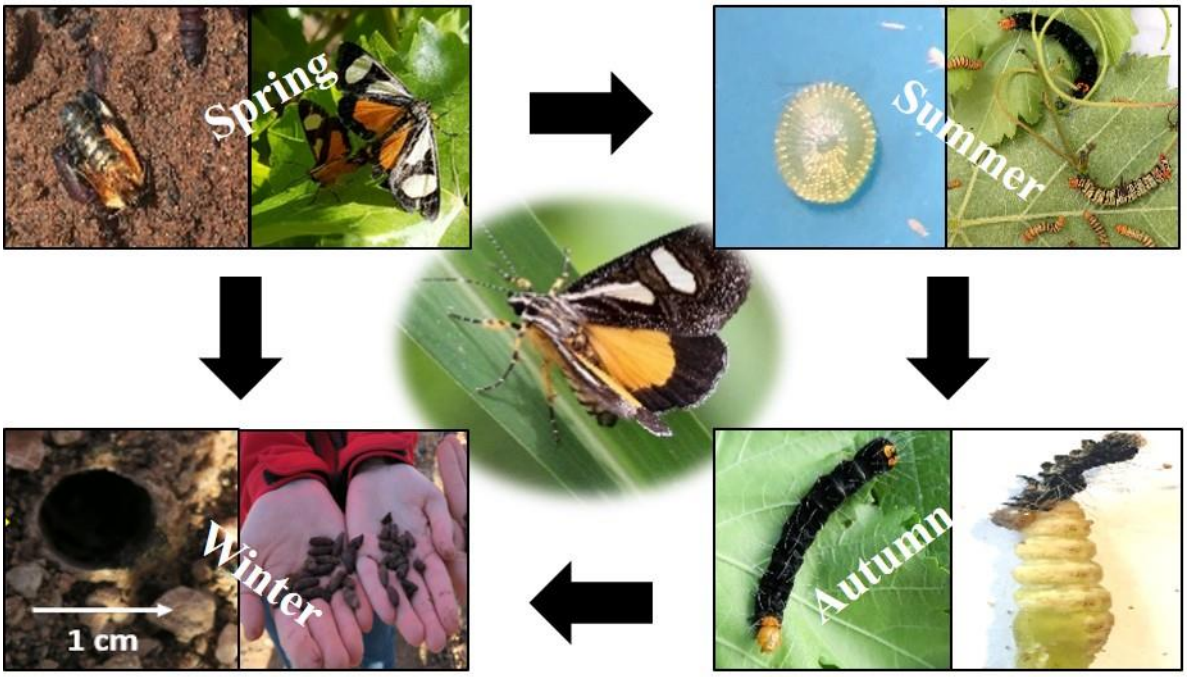

FIGURE 2.3

The general seasonal life cycle of *Agoma trimenii*, from egg to adult.

From observing the presence or absence of the different life stages, it appears that up to four generations of *A. trimenii* may occur in a year (Fig. 2.4). The various life stages of *A. trimenii* overlap at specific times of the year, namely at the end of October, at the end of December, at the beginning of February, at the end of March, and at the end of April. The eggs, which are laid singly on the adaxial surface of leaves (Fig. 2.5), near the top canopy of the vines, hatch after three days (De Waal, pers. comm., 2017).

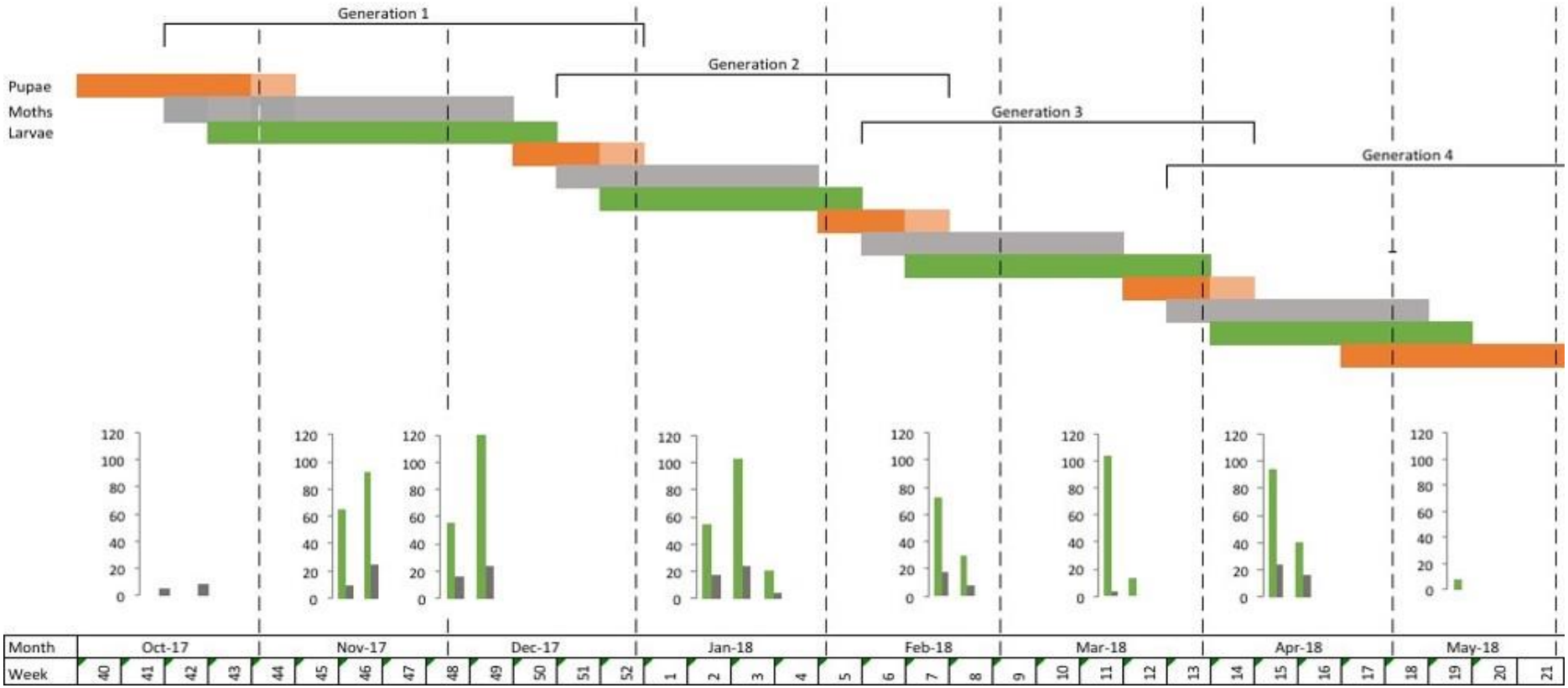

FIGURE 2.4

Presence of life stages of *Agoma trimenii*, as well as larval and moth counts, from October 2017 to May 2018; horizontal bars represent the presence of pupae (orange), moths (grey), and larvae (green) over the monitoring period, and vertical bars represent moth (grey) and larval (green) counts over the 7- to 10-day periods per month.

FIGURE 2.5

Eggs of *Agoma trimenii* on leaf surface.

The length of time for larval development (from hatching to pupation) varies, but can range from 5 to 6 weeks, according to the time intervals occurring from the first presence of the larvae after first moth's flight, to pupation before the next moth's flight (Fig. 2.4). The first instar larvae disperse, beginning to feed on vegetative tissue, particularly on new shoots and leaves. The larvae continue to feed, and to undergo numerous moults (Fig. 2.6A, B), until they reach their final instar. Although larval feeding and adult activity occurs most frequently at night, such feeding was observed during the early morning (± 06:00).

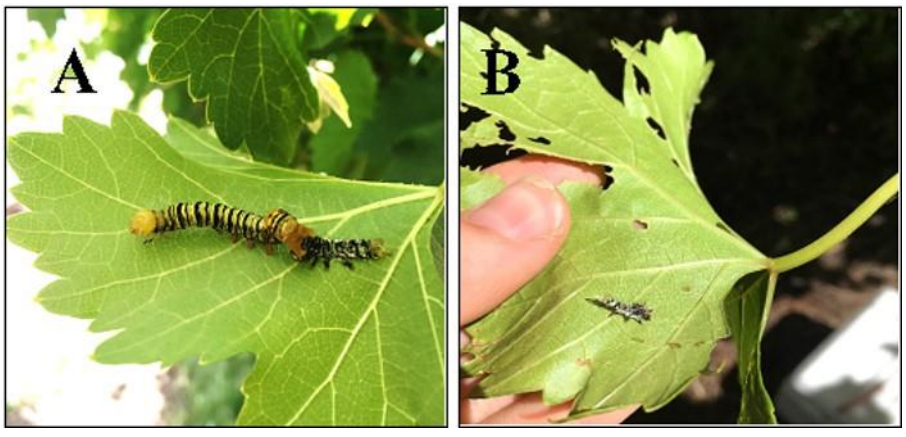

FIGURE 2.6

A: Moulting *Agoma trimenii* mid-instar larva; B: remnants of a shed cuticle of an *Agoma trimenii* larva.

Final instar larvae balloon from the plant, via a silken thread, and burrow into the soil, to a maximum depth of 4 cm in the soil mounds surrounding the vine. They remain in a soft-bodied prepupal state, until they develop into fully formed, hardened pupae. Under laboratory conditions (26ºC, 12:12 L:D and 70 % humidity) it took the prepupae between 4 and 6 h to develop into pupae. The prepupae, which had a yellow/green colouration (Fig. 2.7), were capable of movement. Once the cuticle hardened and turned a brown/red colour, the pupae could, however, no longer move.

FIGURE 2.7

Prepupa of *Agoma trimenii*, and shed cuticle.

Holes in the soil were visible from where the larvae had burrowed, with a diameter of ± 2 cm and a depth of ± 4 cm (Fig. 2.8A). The pupae were found in the soil around the vines, in a radial range of from 15 to 70 cm. Most of the pupae were found closer to the middle of the vineyard block than they were to the perimeter of the vine block. The pupae appeared to remain in the soil for ± 3 weeks before the adult moths emerged (Fig. 2.4). *Agoma trimenii* has a facultative diapause in the pupal stage which carries it through the winter period when the vines are no longer in leaf.

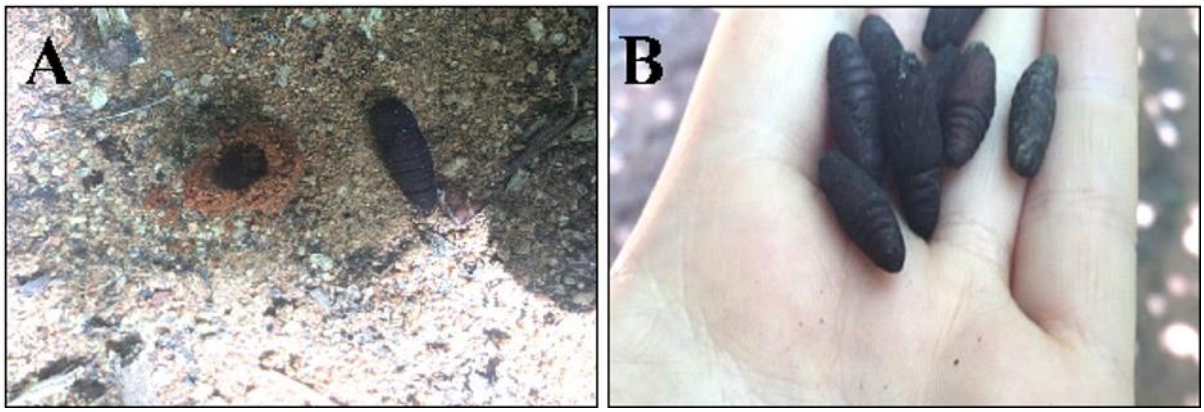

FIGURE 2.8

A: Pupa of *Agoma trimenii*, and hole in the soil where pupation occurred; B: numerous pupae removed from the soil.

Diapause occurred only in the last generation, during May, just before winter. The air temperature at the time averaged 18°C, and the soil temperature at the time on Farm A averaged 14°C. From periodical observations in the quarantine laboratory (DAFF), over 75 % of the stored pupae were found to emerge as adult moths, at 26ºC, 12:12 L:D, and 70 % humidity.

The *A. trimenii* moths emerged from their pupa after ± one week under laboratory conditions (Fig. 2.9). The moths could commence activity shortly after emergence from the pupa. The soft-bodied adults aligned themselves with their heads upwards, and expanded their wings (Fig. 9B). They remained inactive for about an h until the wings had expanded, and the soft cuticle has hardened. Of all the newly emerged moths (n = 23), only two moths were able to survive for longer than two days under laboratory conditions.

FIGURE 2.9

A: Moth of *A. trimenii* emerging from pupa; B: moth of *Agoma trimenii* right after emergence from its pupa.

In the field, peak flight times were recorded towards mid-October, and in mid-December, as well as at the beginning of February, and finally towards the end of March, according to observational studies and visual scouting (Fig. 2.4).

Description of the life stages

The singly laid eggs of *A. trimenii* are circular, with their light-yellow colour against the vine leaves making it almost impossible to see with the naked eye. The eggs become cream-coloured, with irregular brown markings, as they mature (Fig. 2.10).

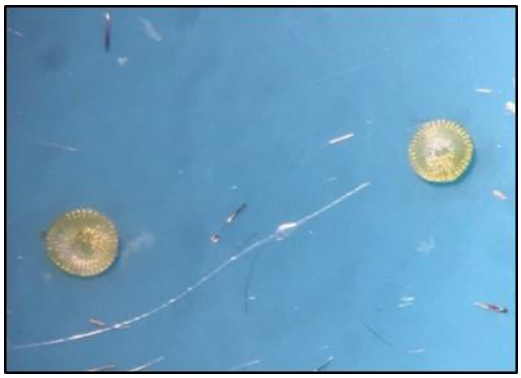

FIGURE 2.10

Eggs of *Agoma trimenii*

When the first instars eclose, the larvae are off-white to yellow/orange in colour, with an orange head capsule, and they measure ± 2 mm. The larvae darken as they feed, later appearing

orange in colour. The subsequent larval instar increases in length (± 3.5 mm), and develops a distinct colouration of black stripes against its orange body (Fig. 2.11).

FIGURE 2.11

Distinct colouration of the larval instars of *Agoma trimenii*.

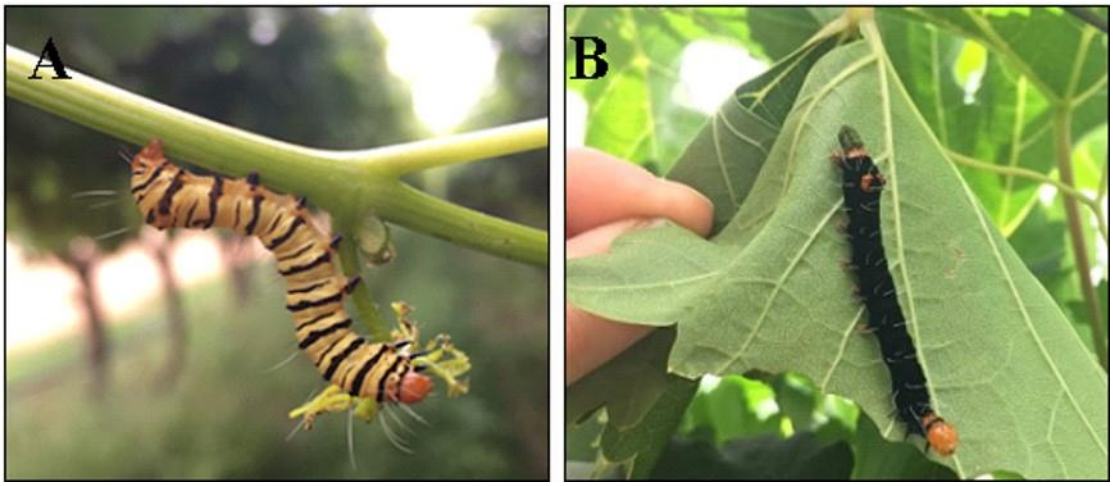

FIGURE 2.12

A: Mid-instar larva of *Agoma trimenii*; B: final instar larva of *Agoma trimenii*.

The later larval instars develop characteristic black and yellow stripes (Fig. 2.12A). The head capsule is a red/orange colour, and the length of the body is covered in small hairs/bristles. The hairs along the length of the body are more striking in the relatively large (later instar) larvae. The rear end of the abdomen has a reddish hump, with two black dots. The final instar measures ± 4.5 cm in body length, with the entire body becoming black, excluding the red head capsule and red hump at the rear end of the abdomen (Fig. 2.12B).

The number of instars is currently unknown, although the larvae of all sizes were collected from the field, and arranged according to size from largest to smallest (Fig. 2.13), so as to give a general idea of the appearance of the different instars.

FIGURE 2.13

Larval stages of *Agoma trimenii*, from latest instar to earliest instar (from left to right).

Agoma trimenii larvae possess toothlike projections on their mandibles, which are used to bite through, and to chew on, the leaves of host plants (Fig. 2.14).

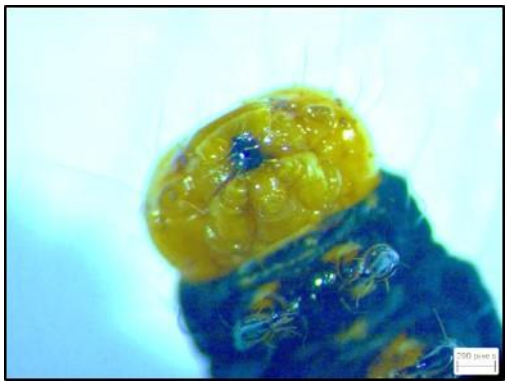

FIGURE 2.14

Mandibles of a final instar larva of *Agoma trimenii*.

The head capsule width of ten differently sized larvae, ranged from largest to smallest, was measured using a microscope. Such measurement provides some indication of the different larval instars, and it might contribute to the determining and the examining of the developmental process from instar to instar. The larval head capsule measurements ranged from 1,900 mm to 4,519 mm (Fig. 2.15A–J).

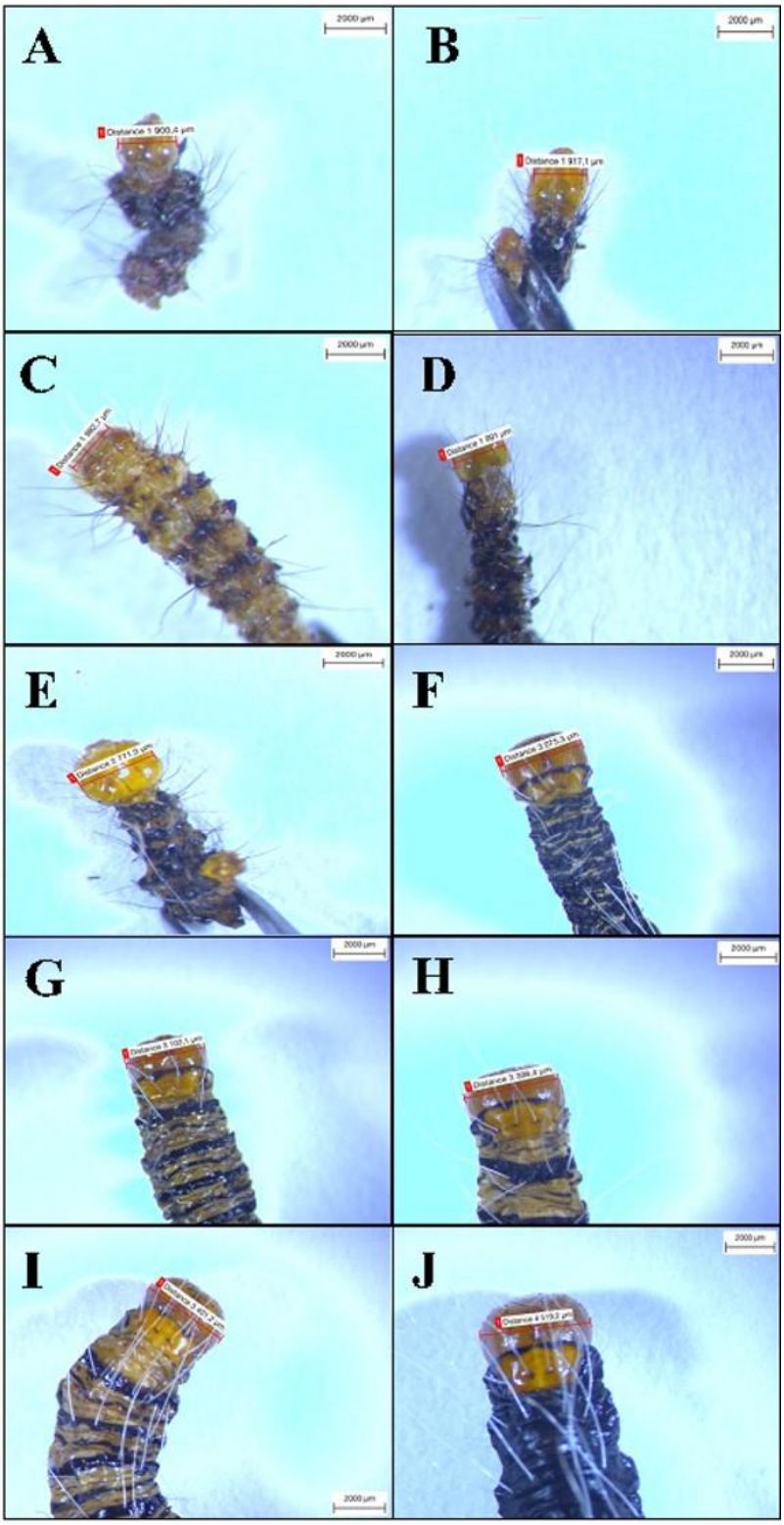

FIGURE 2.15

Head capsule widths: A: 1,900 mm; B: 1,917 mm; C: 1983 mm; D: 1,991 mm; E: 2,771 mm; F: 3,075 mm; G: 3,102 mm; H: 3,398 mm; I: 3,401mm; J: 4,519 mm.

The prepupae of *A. trimenii*, which have a green/brown colouration, are soft (Fig. 2.16). Although it is held that the final instars (black larvae) form prepupae, earlier instars may pupate, if under stress. The above was observed under natural conditions (± 25 ºC), when a larva, measuring ± 2 cm, started pupating once inoculated with a nematode suspension used for experimental purposes, as is described in Chapter 3.

FIGURE 2.16

Final instar *Agoma trimenii* larvae pupating into a prepupa.

The pupae of *A. trimenii* have a hard outer shell and a reddish-brown appearance, which is typical of most noctuids. They darken as they age, eventually becoming dark brown (Fig. 2.17A, B). The mean length of the pupae (*n* = 50) was 1.9 cm, with the mean width being 1.3 mm.

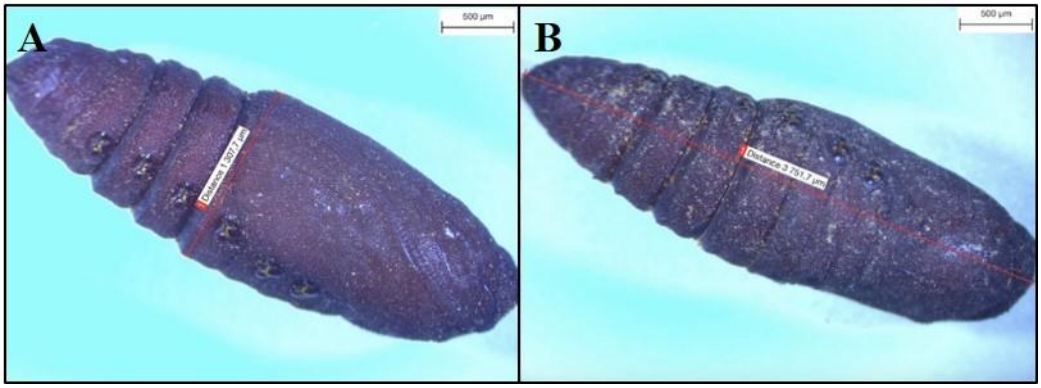

FIGURE 2.17

A: Width of *Agoma trimenii* pupae 1308 µm; B: length of *Agoma trimenii* pupae 3752 µm.

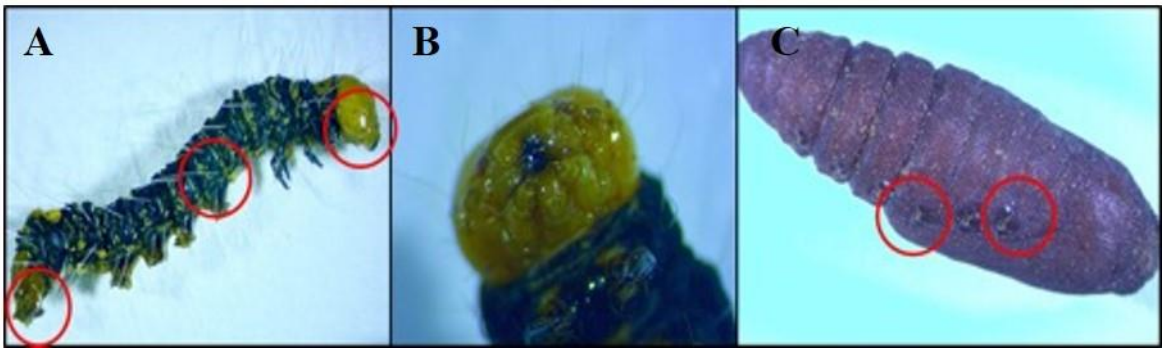

FIGURE 2.18

Natural openings of *Agoma trimenii* larva and pupa, including A: moth, spiracles and anus of *A. trimenii* larvae; B: mouth of *A. trimenii* larva; C: spiracles of *A. trimenii* pupa.

Males and female pupae can be differentiated by characteristic markings on the ventral abdominal terminal segments (Fig. 2.19A, B). Male pupae have triangular markings, while female pupae have rounded markings (Van den Berg, pers. comm., 2017).

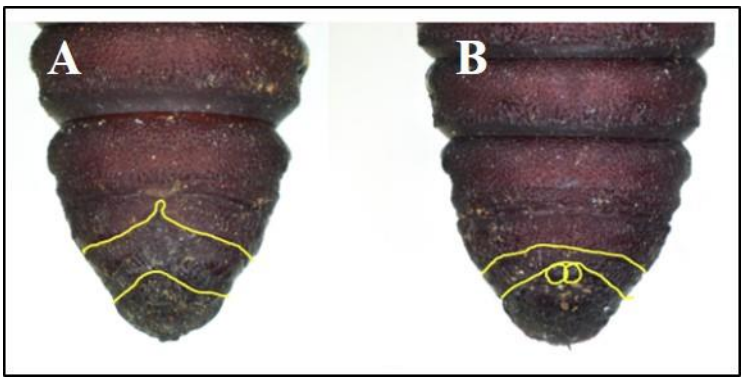

FIGURE 2.19

A: Male pupa; B: female pupa of *Agoma trimenii*.

Adult moths have a wingspan of approximately 5.5 cm. The forewings, which have a black/dark grey border, are black, with two rounded, pale yellow spots at the tip of the wings, and two yellow triangular markings at their base (Fig. 2.20). The hind wings are a light orange colour, with a black border. The abdomen of the moth is orange, with a longitudinal black stripe. *Agoma trimenii* moths have filiform (threadlike) antennae. Male moths appear to be smaller in size than the female moths. The above was observed when the moths emerged from the separated pupae in the quarantine laboratory (DAFF).

FIGURE 2.20

Moth of *Agoma trimenii*.

Behaviour and damage

General observations in the field show that the early larval instars feed on the new shoots and leaves of the vines. As they grow and develop, they feed on the relatively old and large leaves. Larval feeding is most prominent early in the morning, compared to midday feeding, as was observed during the first week of January 2018. The larvae expel a green droplet from the mouth when disturbed, as a possible defence mechanism against predators (Fig. 2.21).

FIGURE 2.21

Mid-instar larva of *Agoma trimenii*, expelling a green droplet.

If disturbed, all the larval stages, excluding the final instar, dropped from the leaves, and hang suspended by a silken thread. The later instars don a characteristic pose when threatened, with the head flung back, so that the thoracic legs are directed forwards. The larvae also display violent curling and uncurling, as well as rapid twisting of their body. The aposematic

colouration of *A. trimenii* larval instars ranging from ± 1 cm to ± 4.5 cm, excluding the black final instars, is difficult to miss, as the bright black and yellow stripes of the body stand out against the vine leaves. The adult moths are nocturnal and most active during the night, although a few of the moths are, sporadically, visible during the day. The moths rest on vine leaves within the canopy during the day, with a few having been noticed resting on the soil mounds surrounding the vines (Fig. 2.22A). Diurnal moth mating was observed (Fig. 2.22B).

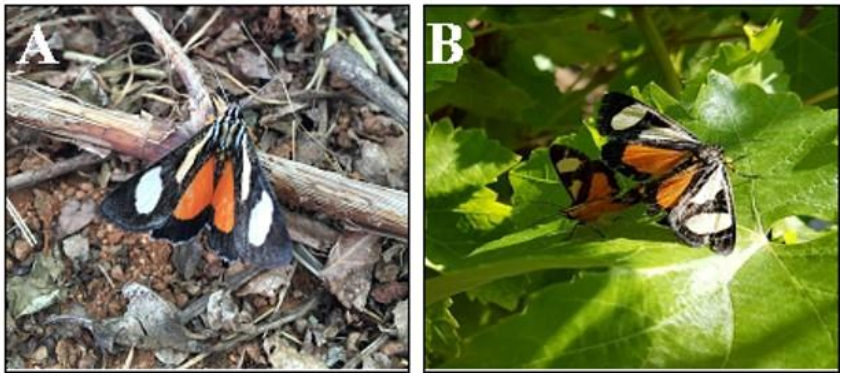

FIGURE 2.22

A: *Agoma trimenii* moth resting on a soil mound; B: mating moths of *Agoma trimenii*.

The larvae appear to be host-specific, feeding on new shoots and leaves. In the current study, the early instars appeared to feed on the shoots and leaves that were highest in the vine canopy (Fig. 2.23A). Foliar damage is most visible from November to January, when temperatures average ± 37°C. All the larval instars, excluding the final instar level, consume most of the young leaf material, including the succulent tissues, veins and midrib (Fig. 2.23 B). Larvae measuring ± 3 cm tend to cause the most severe foliar damage (Fig. 2.23C, D), with them not feeding on the fruit berries. No alternative host plants were found on the surrounding vegetation of the study block.

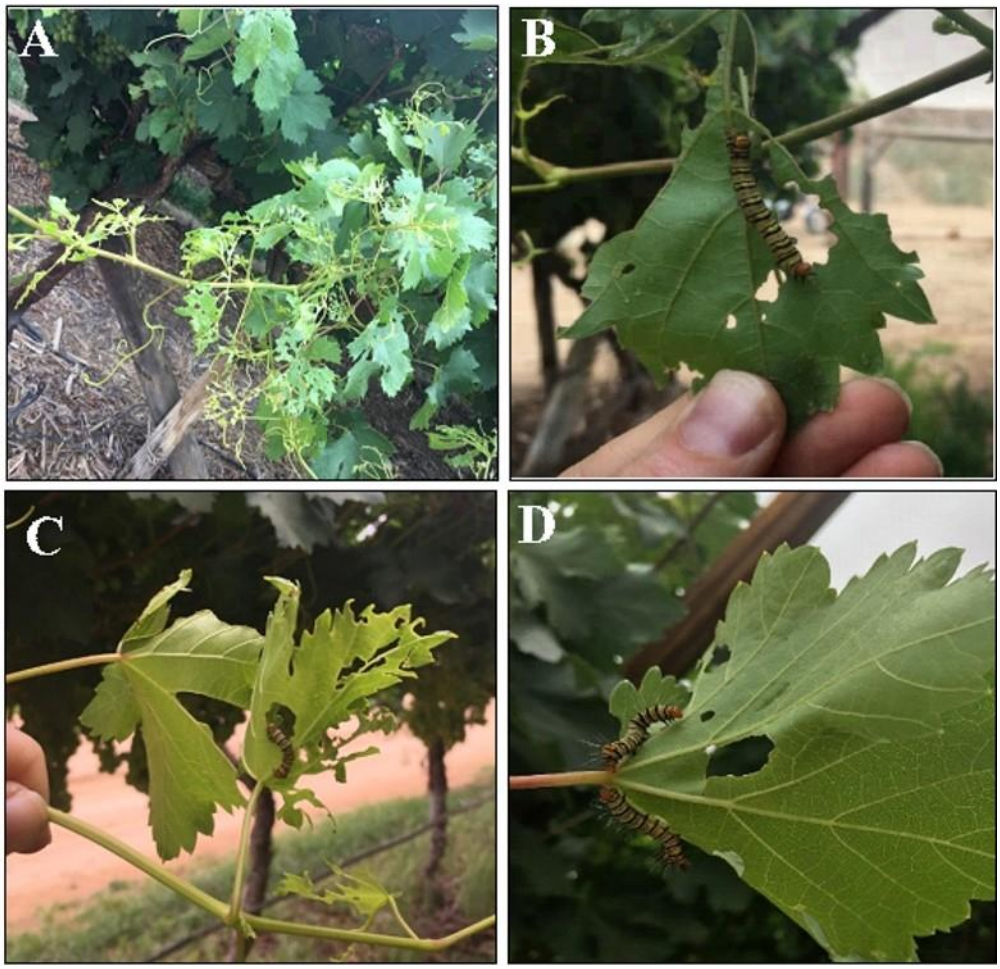

FIGURE 2.23

A: Early instar larval damage to top young shoots and leaves; B: mid-instar larval damage; C, D: examples of leaf damage.

Light trap

Trapping adult moths via an UV blue light trap was found to be the most effective potential monitoring strategy. The maximum number of moths caught per night, by means of the UV blue light on Farm A, was $n = 42$, with the maximum number of moths caught in a night by the solar-powered LED light on Farm B being $n = 12$.

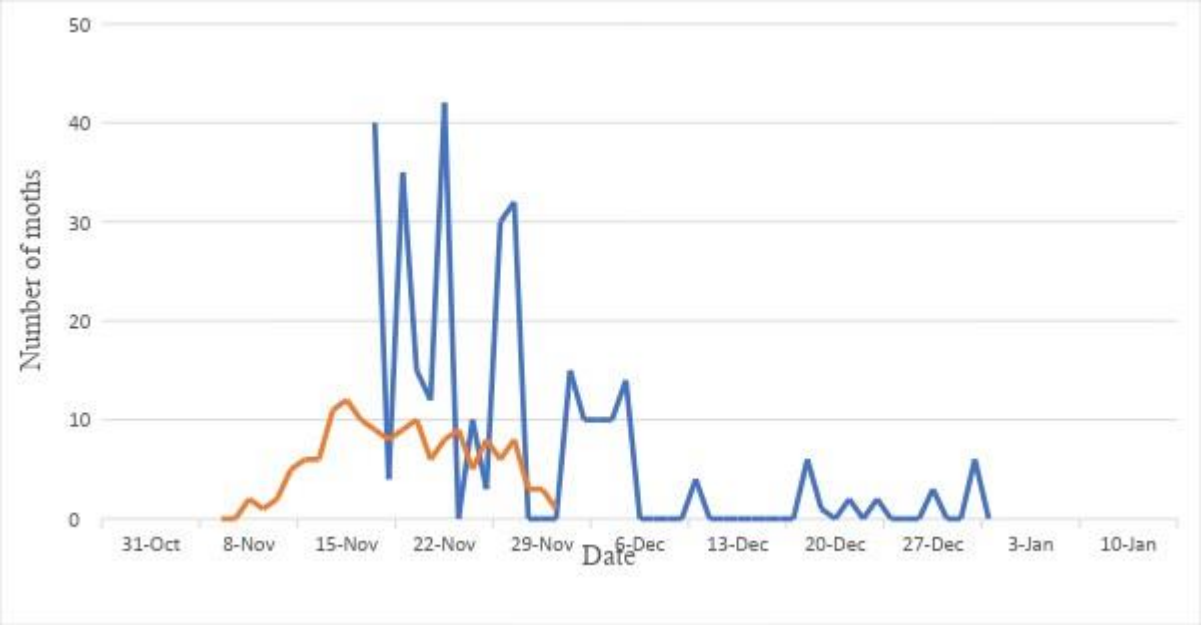

FIGURE 2.24

Graph depicting the number of *Agoma trimenii* moths caught in 2016 at Farm A, using a blue ultraviolet light trap (blue line), and the number of moths caught in 2017 at Farm B, using a solar-powered LED light trap (orange line).

Pheromone and live bait traps

Pheromone traps containing a lure synthesised from isolated semiochemicals of the grapevine moth, *P. glycinae*, were unsuccessful as a potential lure, as no moths were caught on the sticky pads of the yellow delta traps, during the trial period of November 2017. Additionally, no moths were caught at either farm in the cage traps using female virgin moths as lures.

DISCUSSION

The information gained from the present study adds to the general knowledge of the studied pest's behaviour and life cycle. With deepening insight into the seasonal development of *A. trimenii*, as well as of each of its life stages and its behaviour, growers will be able to apply monitoring and control options appropriately, and they will be able to plan effectively to undertake new research projects.

Understanding the seasonal development of *A. trimenii* is crucial to developing a management programme for the species. Biological investigations are important for determining the behaviour of the mobile stages of the moth, and for identifying the periods

when the use of various management strategies might be most effective. From observations made in the field, it is apparent that moth outbreaks commence during mid-October, and that the populations concerned begin to decrease towards April, when the larvae begin to pupate and overwinter. Overwintering can be correlated with low temperatures, as well as with the shortening of day length. All the life stages of *A. trimenii* can evidently be presented simultaneously, with the generations overlapping on more than one occasion.

Understanding and identifying the various life stages of *A. trimenii* sheds light on the extent and timing of foliar damage within vineyards, and it can be used to apply appropriate monitoring strategies and control options. As with numerous other noctuid Lepidoptera, including the grape vine moth, *Phaleonoides glycinae* (Agaristinae), and the Joseph's coat moth, *Agarista agricola* (Agaristinae), the life cycle of *A. trimenii* consists of the egg, most commonly 5 to 6 larval instars, the prepupa, the pupa, and the moth (Cordingley 1980). Descriptions of all the stages of the life cycle should enable the growers to be able to identify *A. trimenii* confidently in their vineyards, and to be assured that the management strategies that they apply are targeting the harmful species concerned. Growers should be aware of the duration of the egg phase and of the time of egg-laying, as such awareness is likely to indicate when they can target newly hatched larvae by means of spraying programmes or other management strategies. The behaviour of early instar larvae is critical to the management of *A. trimenii*. As eggs are laid on the vine leaves, neonate larvae have their food source nearby, so that the larvae can begin feeding relatively quickly after hatching. From observations in the field, it appears that the earliest instars are present on the top leaves and shoots of the vines (± 3 m high). The eggs can, therefore, be assumed to be laid on the highest leaves of the vine. The above indicates to growers that control programmes must consider the positioning of the early instars, if they are to achieve control of the pest, before larval feeding causes extensive foliar damage. If *A. trimenii* is to be targeted for control while it is in the larval stage, management strategies must be timed correspondingly for the period when the neonate larvae are hatching from the eggs. As no eggs were found in the field during the periodic visits that were made throughout the season, the time and duration of the egg phase is still unknown.

Technical information and the determination of larval instars can provide invaluable information for pest management (Castaneda-Vildozola *et al*. 2016; Calvo & Molina 2008). Defining the total number of larval instars can be used to identify the most damaging stages, which is important for control programmes where population management targets the larval

stage (Thakur 2015). From the field observations made, it appears that larvae measuring ± 3 cm in length cause the most foliar damage. Controlling early stage instar larvae is most beneficial, before the leaf damage becomes severe, and the populations increase. The stages are most susceptible to control efforts, owing to their small size and to their movement over the sprayed areas, when searching for new sites on which to feed (Fetting 1999). The above highlights the importance of applying control programmes before the larvae come to measure ± 3 cm. The measurements of larval head capsule width provide basic information for the development of morphometric and ecological studies addressing pest management concerns (Calvo & Molina 2008).

The habit of the Agaristidae family to pupate in the soil (Loch 2005; Pretorius *et al.* 2012) was confirmed in the field, as *A. trimenii* pupae were found in soil mounds around the vines involved. As the pupae of *A. trimenii* are easy to locate, testing possible control options against them is fairly easy in relation to the other life stages. The above-mentioned stage of the life cycle would, ideally, be the targeted stage for control, as the pupae, which are immobile, cause no damage to the vines. Further research on the timing and duration of the pupal stage is required for effective control. Therefore, there is scope to apply control methods against the soil stage, which might include the application of entomopathogenic nematodes (EPNs) and fungi. The soil environment provides sufficient moisture for nematode survival (Rohde *et al.* 2010). EPNs can be applied with almost all commercially available ground or aerial spray equipment, including pressurised sprayers, mist blowers, and electrostatic sprayers (Koppenhöfer 2007). The relevant studies show that targeting lepidopteran pests in the prepupal stage with EPNs (*Neoaplectana carpocapsae)* has displayed excellent results, with the prepupae of both the Beet Armyworm, *Spodoptera exigua*, and of the armyworm, *Pseudaletia unipuncta*, having mortality of 100% (Klein 2018). The softened cuticle might prove to be advantageous in applying EPNs as a possible control option. Locally sourced EPNs, including *H. noenieputen*sis and *S. yirgalemense*, can potentially be effective against the prepupal stage of *A. trimenii*. Therefore, it is of importance to determine the duration of the stage of the life cycle identified, so as to be able to apply the appropriate control methods. However, the duration of the prepupal stage is short, based on observation, and identifying the exact time of pupation, as well as applying control methods against the prepupal stage, could prove to be problematic.

The moths of *A. trimenii* are easy to identify, owing to their characteristic markings and colouration. It is, therefore, possible to identify the first generation of *A. trimenii*, as the moths can be observed flying around vineyards during the day. Additionally, their nocturnal activity can easily be monitored by means of light traps, which will be discussed later. The sex ratios of *A. trimenii* moths in the field have yet to be established. Establishing sex ratios can track the differential emergence of both sexes throughout the season (Knight & Light 2005).

The different larval stages of *A. trimenii* display various behaviours that might serve as defence mechanisms. The evasive behaviour of all the larval instars of *A. trimenii* includes thrashing and twisting. The aposematic colouration of the larvae could be important for visual predators. The predators being repelled by brightly coloured prey provides support for the long-standing belief that aposematism is an adaptive explanation for bright colouration and striking patterns (Greeney *et al.* 2012). The red hump on the last abdominal segment of the mid- to late instars may cause potential predators, like birds, to mistake the tail for the head, and, due to the former being larger than the latter, the prey might repel their predators, or they might divert their attention away from the more vital head region. The expulsion of a green droplet from later instars is known as regurgitation, and, although the toxic properties of regurgitates, and their level of efficacy against enemies, is largely unknown for most species, most, or all, have been found to have repellen, or deleterious, effects on their potential enemies, and are likely to be toxic to some degree (Greeney *et al*. 2012; Oliveira & Freitas 2004). The attributes and behaviours concerned, including regurgitation and dropping via silken threads, all suggest that *A. trimenii* might have potential natural predators in their places of origin, although no predation was observed in the current study.

Of all the monitoring methods tested in the present study, including light traps, pheromone traps, and live bait traps, light trapping proved to be the most successful strategy, with the added benefit of doubling up as a mass control option.

Light trapping is regarded as a successful monitoring strategy for noctuid moths, as they are most active during the night time, and exploit their attraction to artificial light (Jonason *et al*. 2013). Light trapping has become a standard, and widespread method in ecology, taxonomy, and lepidopteran monitoring strategies, with it being represented as the only method allowing many species to be sampled quantitatively in large numbers (Holloway *et al.* 2001). Light traps provide information on the presence or absence of a species, on mapping distribution and on the clarification of phenology, by enabling the determination of flight periods (New 2004). By

consistently and periodically trapping moths via light traps, it is possible to time the emergence of pest moths, and, subsequently, to establish appropriate spraying programmes, according to the seasonal development of the moth. The main advantage of using light traps is the large number of individuals that can be recorded during a relatively short period of time (Van Langeveld *et al*. 2011). Light traps can be designed in several ways, and they can be operated using different light sources. Both such modifications are known to affect trap performance (Fayle *et al*. 2007; Intachat & Woiwod 1999). Light sources with a high proportion of UV radiation tend to attract comparatively more individuals than might be attracted otherwise, as well as other taxa (Van Langeveld *et al*. 2011). The above is evident in the experimental procedure adopted, as the moths concerned were evidently more attracted to the blue UV traps than to the LED solar-powered light traps, when considering the maximum number of moths that might be captured in a night. Trapping moths using the appropriate light traps not only enables the effective monitoring of moth populations, but it can also serve as a mass trapping technique, which constitutes a potential control option (Shimoda & Honda 2013). According to Muirhead-Thompson (2012), no other trapping technique has proved so consistently effective in capturing larger numbers of a wide variety of species. In fact, the light-trap captures of some pest species have been so extraordinarily high as to lead to the attempted control of pests, including cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), and tobacco hornworm, *Manduca sexta* (Sphingidae), by means of a system of traps alone, with the aim of producing a significant decrease in the populations concerned (Hartstack *et al.* 1968).

Recent studies (Jonason *et al*. 2014) showed that mercury vapour lamps are more effective at attracting the moths of various species than are UV inflorescent lamps, with 29 953 individuals, representing 334 species, and 19 519 individuals, representing 299 species, having, respectively, been caught by such light traps respectively. It would, therefore, be useful for growers if the effectivity of mercury light traps were compared to that of UV blue light traps in vineyards, specifically for the monitoring and control of *A. trimenii* moths.

The advancement of synthetic insect pheromones has provided the agricultural industry with a highly effective tool for the early detection of pest infestations (Mullen & Dowdy 2001). The use of pheromone-baited traps to monitor insect populations offers several advantages over the undertaking of visual inspections, with the foremost among them being that traps work 24 h a day, and 7 days a week. Maintaining of traps is simple, with it requiring regular inspection for

the recording of the numbers and species of insects caught, so as to clean the traps, and to replace the lures (Mullen & Dowdy 2001). As the pheromone lure synthesised from isolated semiochemicals of the grapevine moth, *Phaleonoides glycinae*, used in the current study (with a 50:50 combination of 2-phenylethanol and phenethyl acetate) was unsuccessful in attracting *A. trimenii* moths, it cannot be used for monitoring and control purposes. However, it is possible that the lure required for *A. trimenii* is specific in terms of its own semiochemicals as a lure. Research into the presence of a pheromone, and subsequent isolation, synthesis and evaluation, is required before trapping can be considered in *A. trimenii*-infested vineyards. Koppert Biological Systems (a company producing sustainable cultivation solutions for food crops), which is based in the Netherlands, is currently in the process of synthesising the *A. trimenii* pheromone lure. Future research and application of the lure might result in monitoring and control options. Field observations suggest that pheromone traps for the capture of *A. trimenii* be placed in the field during early October, before the first post-winter peak in moth activity. Trapping using live virgin female moths as lures is an outdated method, with studies dating back decades on the monitoring of cabbage looper moths, *Trichoplusia ni* (Lepidoptera: Noctuidae), and spruce budworms, *Choristoneura* (Lepidoptera: Tortricidae), having been undertaken by Birch (1977) and Miller & McDougall (1973), respectively. Replacing female moths obtained from laboratory colonies on a weekly basis, as was carried out by Bethell *et al.* (1972) was problematic for the present study, as a colony of *A. trimenii* would have been required.

As with many research programmes, constraints restricted the scope and outcomes of the present study. However, recommendations, both in the field and for future research, are provided for the future guidance of how to deepen the current understanding of the pest. Visual scouting should be done at longer timed intervals over the entire growing season. Although the above could be focused on vineyard hot spots, spreading scouting across the vineyard would is useful to come to know where the moth activity is greatest (Jordan 2014).

The quarantine status of *A. trimenii* limited the transportation of larvae and moths to suitable laboratories in the Western Cape. The rearing of *A. trimenii* populations in captivity could allow for the accurate timing of all life stages, as well as of survivorship, the developmental times of all life stages, and the behavioural traits concerned (Zagorinskii *et al*. 2013). Outstanding aspects regarding the biology of *A. trimenii*, such as the exact times of overlapping generations, could be resolved by reporting on the observations of *A. trimenii* colonies in

captivity, reared on an appropriate diet, and under suitable environmental conditions, in relation to the habits of those surviving in the field. Additionally, rearing a colony could provide information on the larval instars involved, which could prove to be imperative for mortality–survivorship research undertaken on the basis of life tables, as well as that which is undertaken in terms of population modelling (Castaneda-Vildozola *et al.* 2016). The above emphasises the need for further research to be undertaken into the determination of different larval instars using two possible methods, being the inspection of a frequency distribution of the measurements of head capsule width, and the checking of a bivariate plot of mean instar sizes against the presumed instar number (Delbac *et al.* 2010). The results obtained therefrom would, further, stand to contribute to the understanding of the developmental stages of *A. trimenii*. In the current study, the moths that were reared in the quarantine laboratory did not survive for longer than two days, as a possible result of the unsuitable conditions prevailing. The average humidity was found to be far greater in the laboratory (70 %) than it was in the field site from which they were collected (31 %) during the 2017 summer season. The above should be taken into consideration if a colony of *A. trimenii* is reared in captivity in future.

A suitable pheromone lure for *A. trimenii* can be used as a potential monitoring strategy. The prospect of being able to use a lure that is specific to *A. trimenii* holds tremendous value for future efforts in terms of creating a monitoring system that is based on pheromone traps, as well as in relation to devising a control programme using the pheromone lures for possible mating disruption. Synthetic chemicals could be produced in large amounts to confuse the males and to limit their ability to locate the females (Suckling *et al*. 2014). In addition, a delay in mating, by means of reducing the number of available males, may also assist in population control, as has already been presented in numerous mating disruption studies (Fraser & Trimble 2001; Jones *et al.* 2008; Stelinski & Gut 2009). Population monitoring relates trap captures to the abundance of, or to the damage caused by, a pest species (Abrol & Shankar 2012). The size of trap captures is used to establish thresholds, either for the timing of control procedures, or for deciding whether it is necessary to take remedial action (Witzgall *et al.* 2010). Pheromone trap counts can be used to calculate a degree–day spray-timing model, involving adding degree–day summations, beginning on the date of the first catch in pheromone-baited traps for each generation, and continuing until an experimentally determined sum has been caught (Fetting 1999). The sum, which specifies the optimal spray date for each generation, is based on moth phenology. The degree–day calculation should indicate to growers when egg hatching

will occur, and when the next generation of the pest should be beginning to fly (Knutson & Meugge 2010).

The above-mentioned recommendations shed light on certain parameters of the life cycle and biology of *A. trimenii*, including on the duration of each life stage, the timing of overlapping generations throughout the life cycle, the timing of peak moth flights, and desirable methods of control.

CONCLUSION

The Trimen's false tiger moth is of increasing economic importance in some grape-producing regions of South Africa, and further research into its biology is crucial for adopting appropriate monitoring strategies and methods to control the increasing populations. Results from the current study shed light on the biology and seasonal development of *A. trimenii*, which could form the groundwork for future research. The setbacks of the study that were previously addressed affirm the necessity for the adoption of suitable monitoring methods for *A. trimenii*, for obtaining more information than is presently available on the flights and mating periods concerned, and for correlating the data obtained with the existing biological, physical and environmental parameters. Light trapping by means of UV blue, which is currently the most promising, and the easiest, method of monitoring *A. trimenii*, could also be used for the mass-capturing of *A. trimenii* as a control option. Light trapping, combined with a suitable monitoring strategy, should provide the growers with accurate information for the appropriate timing of pesticide applications. To time the spraying of applications for *A. trimenii*, the monitoring of traps and the weekly scouting of vineyards could be used to ascertain when the moths and larvae are most active. To achieve low-pest densities, pest populations should be detected while they are in the early stages of infestation, and before serious problems have a chance to develop. Knowledge and the development of the resulting economic threshold levels and the injury levels of suitable monitoring strategies should aid in the appropriate control of *A. trimenii*. Incorporating different monitoring and control strategies might contribute towards maintaining an integrated pest management system in relation to the new and potentially devastating pest in South African vineyards, namely Trimen's false tiger moth, *A. trimenii*.

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Chapter 3

Laboratory Bioassays on the Susceptibility of the Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae), to Entomopathogenic Nematodes and Fungi

ABSTRACT

Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae), is an economically important pest of vineyards in the Northern Cape province, South Africa. As little research on the biology of *A. trimenii* exists, control options are lacking. The aim of this study was to test the susceptibility of larvae and pupae to entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae and two commercially available entomopathogenic fungal (EPF) isolates under laboratory conditions. The pathogenicity of two local EPN species was screened against larvae and pupae, using a concentration of 100 infective juveniles (IJs) in 50 µl of water. After 48 h, 100% mortality of the larval stage was found. However, in the case of the pupae, no infection with EPNs was observed. The pathogenicity of two EPF isolates, *Metarhizium anisopliae* and *Beauveria bassiana*, was screened against larvae and pupae by means of a dipping test undertaken at a concentration of 0.2 ml/500 ml water and 0.5 g/500 ml water, respectively. At 15 days post treatment, 100% larval mortality was recorded. However, no mortality of the pupae was observed. The prepupal stage should be screened for susceptibility to EPNs and EPF in future research. The results indicate good potential for EPNs and EPF as biological control agents only against the larvae of *A. trimenii*, within an integrated pest management system.

Key words: *Agoma trimenii*, biological control, Trimen's false tiger moth, entomopathogenic fungi, entomopathogenic nematodes

INTRODUCTION

Trimen's false tiger moth, *Agoma trimenii* Felder (Lepidoptera: Agaristidae), is an indigenous lepidopteran that is classified within the Noctuidae family and the subfamily Agaristinae. The moth recently developed pest status in parts of South Africa, after reports of moth infestations in vineyards in the summer rainfall areas (Pretorius *et al*., 2012). *Agoma trimenii* is considered a severe agricultural pest, targeting the young shoots and leaves of wine, table and raisin grapes in the Northern Cape and Limpopo provinces of South Africa. As the economic importance of *A. trimenii* in the grape-producing regions of South Africa increases, so does the need to control its increasing population.

To date, control measures entail the repeated application of chemical insecticides against *A. trimenii* larval infestations. Such insecticides, which are currently undergoing registration, may threaten to induce the development of secondary pests that are currently under natural control (Pretorius *et al*., 2012). Despite the suppression of pest populations with insecticides, undesirable effects are often unavoidable; insects that are genetically resistant to chemicals are usually selected, and non-target organisms, including natural enemies of the pest, can be wiped out (Blomefield, 2003; Gullan & Cranston, 2014). Pesticides then used to reduce the level of natural biological control agents (Wakgari & Giliomee, 2003). Consequently, pest resurgence can potentially exceed the levels found prior to insecticide treatment, if the natural predators recover at a slower rate than does the pest population (Gullan & Cranston, 2014). Secondary pest outbreaks are also potential setbacks that lead to insect species that were previously not regarded as pests being released from control, and potentially becoming major pests (Mgocheki, 2008). Concerns over human health (either directly, from the handling and consumption of insecticides, or indirectly, via exposure to environmental sources) and environmental impacts have encouraged the development and utilisation of alternate pest management programmes, products and technologies (Blomefield, 2003). Integrated pest management (IPM) strategies address such concerns and accommodate new pest control techniques (Ahmad & Kamarudin, 2011).

Biological control involves the making of deliberate human intervention efforts to re-establish the ecological balance (of the abundance and distribution of species), by means of introducing, or intensifying, enemies in terms of their host specificity. However, the enemies involved, unfortunately, do not completely eliminate the pests concerned (Chidawanyika *et al*., 2012). Therefore, biological control does not alleviate all the economic consequences of pests,

but control systems are expected to diminish the abundance of a target pest to below the prevailing economic threshold levels (Gullan & Cranston, 2014). Potential biological control strategies for *A. trimenii* include the application of entomopathogenic nematodes and fungi. Such biological control agents exhibit many beneficial traits, including offering minimal risk to human health, the absence of toxic residues in crops, minimal risk to beneficial and other non-target insects, and host specificity (Inglis *et al*., 2001; Goettel *et al*., 2005). Testing the above-mentioned biological control options on *A. trimenii* is important, as no such control options currently exist.

Entomopathogenic nematodes (EPNs) belonging to the *Heterorhabditis* and *Steinernema* families, which are found in soils throughout most parts of the world, are parasitic to a broad range of insect pests (Malan & Hatting, 2015). Both families have a unique symbiotic association with the entomopathogenic bacteria, *Photorhabdus* and *Xenorhabdus*, and, together, can successfully parasitise and kill their insect hosts (Ehlers, 2001; Jang *et al*., 2011). Upon encountering a suitable host, the free-living and non-feeding infective juvenile (IJ) enters the host insect through its natural openings, like the mouth, the spiracles, or the anus (Griffin *et al*., 2005; Gözel *et al*., 2015). The bacteria rapidly replicate within the nutrient-rich haemolymph of the host, generating various toxins, as well as a variety of primary and secondary metabolites that kill the host by means of inducing lethal septicaemia within 48 h of infection (Jang *et al*., 2011; Le Vieux & Malan, 2013; Griffin *et al*., 2005).

The advantageous attributes of *Heterorhabditis* and *Steinernema* species for effective biological control include high virulence and the ability to seek out well-hidden hosts actively (Lacey & Georgis, 2012; Malan & Ferreira, 2017). Additionally, they are compatible with commercial rearing and application techniques (Shapiro-Ilan *et al*., 2012). The entomopathogenic activity of both steinernematids and heterorhabditids has been documented against a broad spectrum of insect pests in diverse habitats in South Africa (Hatting *et al*., 2009; Malan *et al*., 2011). However, their effectivity against *A. trimenii* has yet to be tested.

Entomopathogenic fungi (EPF), including *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschnikoff), are ubiquitous microorganisms that attack a variety of arthropods by means of inducing acute mycosis (Barta, 2010). The EPF can disperse rapidly horizontally among host populations by means of aerially produced conidia, and by infecting their host through penetration of the cuticle with germ hyphae (Bidochka & Small, 2005; Barta, 2010). Identification of the EPF isolates relies generally on their physical appearance on culture

media. Morphologically, the conidia spores of *B. bassiana* are a powdery cream colour, turning yellow in colour with age, whereas *M. anisopliae* is varying shades of green (Coombes, 2012). The virulence of both *B. bassiana* and *M. anisopliae* is mainly a factor of the ability of the conidia to penetrate the insect's cuticle. Death of the insect host can then proceed, as a result of a combination of such effects as toxinosis, general obstruction due to hyphal growth, and nutrient depletion (Wraight *et al.*, 2007). Previous studies on the effect of EPF on host insects like the cotton aphid, *Aphis gossypii* (Glover) (Hemiptera; Aphididae), the noctuid tobacco caterpillar, *Spodoptera litura*, the sweet potato weevil, *Cylas puncticollis*, and the red palm weevil, *Rhynchophorus ferruginueus*, show that EPF may influence the host insect through behavioural and feeding changes, reduced body weight or fecundity, and malformations (Gindin *et al*., 2006; Ondiaka *et al*., 2008; Malarvannan *et al*., 2010; Gurulingappa *et al.*, 2011). Both fungal species are considered safe to vertebrates, and, although they are known to have a wide host range, different strains tend to have restricted host ranges, making them suitable for use in biological control programmes (Zimmermann, 2007a,b; Hatting *et al*., 2009). Additionally, the species concerned are easy and relatively inexpensive to cultivate on artificial media, which is an advantageous trait in respect of commercialisation (Kaya & Lacey, 2007). Both fungal species are known to target and successfully infect the larval and pupal stages of numerous lepidopteran pests (Nguyen *et al.*, 2007; Coombes, 2012; Oliveira *et al.*, 2012). However, their effectivity against *A. trimenii* has yet to be tested.

The current study investigated the potential of EPNs and EPF to infect *A. trimenii* under laboratory conditions, and determined their susceptibility for use as part of an IPM programme directed at the management of the pest. Biological assays are the starting point of any biological investigation in which virulence is of importance, as they allow for the removal of factors that could reduce virulence towards the target host. Laboratory bioassays were performed against the larval and pupal stages of *A. trimenii* to determine the potential of EPNs and EPF as biological control agents against the identified stages of the insect concerned.

MATERIALS AND METHODS

Source of pupae and larvae

Both the pupae and the larvae of *A. trimenii* were collected from demarcated field sites on two table grape farms in the Northern Cape province, South Africa. Both sites consisted of netted blocks of table grapes. On Farm A (28°39′52.4″S 21°07′52.8″E) the block was made up of the

Thompson Seedless table grape variety, whereas on Farm B (28°67′84.4″ 20°39′59.9″E) the variety was Sugraone. The pupae were collected during the winter months of May 2017 and June 2018. Soil mounds surrounding the vines were excavated to a depth of ± 5 cm and a radius of ± 60 cm. The pupae, which were stored in a thin layer of moistened soil in 2-L plastic containers, were kept in a growth chamber at 25°C. Various larval instars were collected in January 2018 from the top and bottom leaves of the vines concerned. The larvae, which ranged from 1.5 cm to 4.5 cm in length, were stored in plastic 2-L containers, provided with a mesh-covered hole in the lid so as to ensure adequate ventilation within the container, and they were given vine leaves on which to feed. The containers were stored under conditions similar to those found in the natural environment.

Source of nematodes and fungi

The local EPN species, *Heterorhabditis noenieputensis* Malan, Knoetze & Tiedt and *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams, used in the study were obtained from previous surveys and stored in Stellenbosch University's nematode collection (Malan *et al*., 2006, 2011). Infected juveniles (IJs) of the two species were cultured *in vivo*, making use of the last instar of the greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), at room temperature (Griffin *et al*., 2005). The rearing, harvesting and quantification of the IJs of both nematode species was conducted according to the methods described by Kaya & Stock (1997). Within the first week of emergence, the IJs were harvested and stored horizontally in vented 500-ml culture flasks, containing approximately 100 ml distilled water. The nematodes were used within one week after harvesting. The nematode concentrations that were used against the pupae and larvae, respectively, were calculated using Navon & Ascher's (2000) equation.

Two commercial fungal isolates, *Beauveria bassiana* (EcoBb strain R444) and *Metarhizium anisopliae* (ICIPE 69), were received from the respective South African manufacturers, Plant Health Products and Real IPM. Concentrations of both products were used, following the guidelines stipulated on the respective labels.

Bioassay protocol for entomopathogenic nematodes

Pathogenicity screening was conducted using 6-well bioassay plates, with each being lined with filter paper (25-mm-diam.). Each well of the bioassay plates was inoculated with 50 µl of

an adjusted IJ suspension, using a Pipetman® micropipette. A single insect was added to each inoculated well (Fig. 3.1), which was then covered with a lid. An identical replicate control for each treatment was prepared on the day of screening, and 50 µl of distilled water only was added to each well. The bioassay plates were then placed in 2-L plastic containers, lined with moistened paper towels and closed with a lid, to ensure the maintenance of high levels of humidity (RH ± 95%). The containers were kept in the dark at 25 ± 2°C for 48 h. After 2 days, the insects were removed from the inoculated well plates and examined.

FIGURE 3.1

Test arena for the screening of *Agoma trimenii* pupae (as well as the other stages) against entomopathogenic nematodes and fungi.

Pathogenicity of entomopathogenic nematodes

Pupae

The pathogenicity of *S. yirgalemense* and *H. noenieputensis*, to the pupal stage of *A. trimenii*, was tested at a concentration of 200 IJs/50 µl per pupa, using the bioassay protocol described above. For each EPN isolate, five 6-well bioassay plates were used (n = 30), together with replicate controls for each treatment (n = 30). After 48 h, each pupa was held against a heated hotplate for ± 15 secs, to assess its mortality by observing any movement that it made in response to the heat. The pupae that showed movement were considered alive and uninfected, while the pupae that showed no movement were dissected and examined under the microscope for nematode infection. The experiment was repeated on a different test date with a fresh batch of nematodes, resulting in two replicate tests for each tested EPN species.

Larvae

The pathogenicity of *S. yirgalemense* and *H. noenieputensis* to the larvae of *A. trimenii* (at various stages of development) was tested at a concentration of 100 IJs/50 µl per larva, according to the bioassay protocol described. For each EPN isolate, four 6-well bioassay plates were used (n = 24), with a control per EPN isolate being prepared (*n* = 24) on the day of screening. The pathogenicity was recorded by means of evaluating the mortality caused by nematode infection by both the isolates. After the 48 h exposure period, the larvae were rinsed with distilled water, and the dead specimens were transferred to clean Petri dishes (90-mm-diam.) lined with filter paper, and moistened with 800 µl of distilled water. The Petri dishes were sealed with PARAFILM® and placed in a dark growth chamber for a further 48 h, so as to encourage nematode development. The number of IJs that successfully penetrated each larva were counted, after the dissection of each host (Fig. 3.2) using a stereomicroscope. To determine the percentage of penetration, the number of IJs counted was divided by the concentration (i.e. 100 IJs/larva) concerned. The experiment was repeated with a fresh batch of nematodes on a different test date.

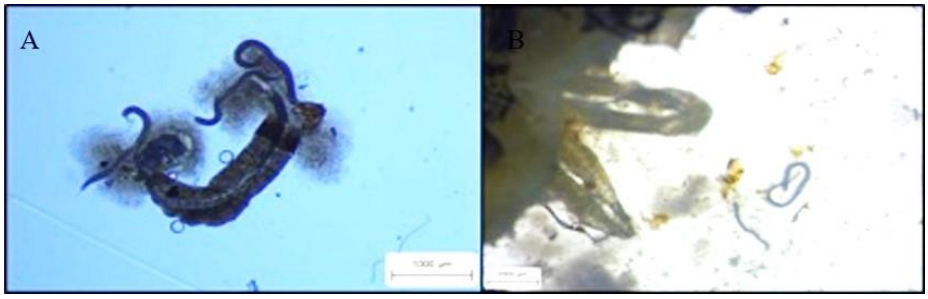

FIGURE 3.2

A*: Agoma trimenii* larva dissected after infection with 100 IJs/50 µl per larva of *Steinernema yirgalemense;* B: *Agoma trimenii* larvaa infected with 100 IJs/50 µl per larva of *Heterorhabditis noenieputensis*.

Bioassay protocol for fungi

The virulence of both EPF species, *B. bassiana* and *M. anisopliae*, to the pupae and larvae was assessed by means of a dipping bioassay. A conidial suspension of EcoBb (*B. bassiana* formulation) and Met69 (*M. anisopliae* formulation) was prepared by adding 0.5 g of EcoBb and 0.2 ml of Met69 to 500 ml distilled water, respectively. The insects were dipped in

respective 500-ml conidial suspensions for 30 sec. Excess suspension was removed by waiting for any surplus droplets to fall. 6-well bioassay plates lined with filter paper were used to conduct the bioassays. The dipped insects were placed onto the filter paper of each well, and water was added to the filter paper (with no free water being allowed to remain), depending on the level of moisture obtained from each dipped insect. As controls, the insects were dipped in distilled water before being placed in their respective wells. The bioassay plates were placed in 2-L plastic containers, lined with moistened paper towels, and closed with a lid, so as to ensure the maintenance of high levels of humidity. The containers were kept in the dark at 25 ± 2°C for 5 days. After the set period of time had elapsed, the insects were removed from the bioassay plates and cleaned of external fungi by being dipping separately into a series of six Petri dishes containing 5% bleach, distilled water, and a 70% ethanol solution, followed by an additional three Petri dishes containing distilled water. Each dip lasted for 30 s.

Pathogenicity of entomopathogenic fungi

Pupae

For the virulence screening of each EPF isolate, five 6-well bioassay plates were used (n = 30), with the control pupae (n = 30) following the bioassay protocol for the fungi described above. After 5 days of exposure, and subsequent washing, the pupae were transferred to 90-mm-diam. Petri dishes containing a selective medium of Sabouraud Dextrose Agar (SDA) to encourage mycosis (Fig. 3.3). After 10 days of possible infection, the pupae were examined visually for fungal growth. The mycosis of both isolates was recorded by observing the characteristic coloration of the conidia on the agar plates (with *B. bassiana* displaying a white mass of conidia spores, and *M. anisopliae* displaying a green mass of conidia spores). Mortality was assessed by holding each individual pupa against a heated hotplate, and observing any signs of movement. The pupae responding to the emitted heat were considered to be uninfected.

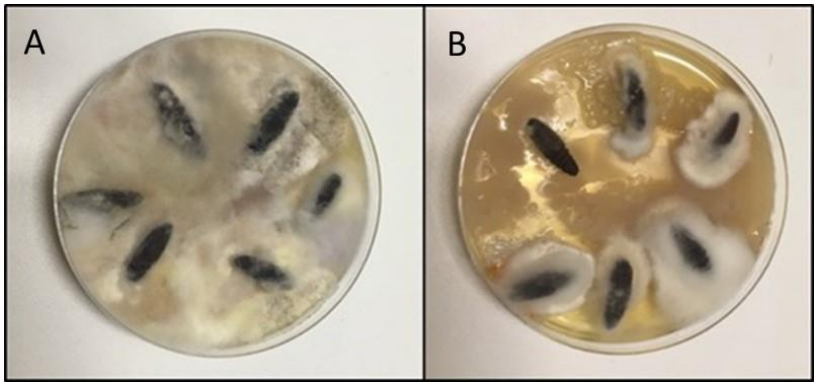

FIGURE 3.3

A: Sabouraud Dextrose Agar (SDA) plates with *Agoma trimenii* pupae screened with *Metarhizium anisopliae* at a concentration of 0.2 ml/500 ml distilled water: B: Sabouraud Dextrose Agar plates with *Agoma trimenii* pupae screened with *Beauveria bassiana* at a concentration of 0.5 g/500 ml distilled water.

Larvae

The virulence of both EPF isolates to the various instar levels of *A. trimenii* larvae was evaluated by means of following the dipping bioassay protocol, as described. Four 6-well bioassay plates (n = 24) and an identical control (n = 24) were used per EPF isolate. After 5 days of exposure to EPF, dead larvae were removed from the bioassay plates and transferred to Petri dishes containing SDA, so as to encourage mycosis. The Petri dishes were sealed in 2-L plastic containers, which were lined with moistened paper towels, and then placed in a growth chamber of 25 ± 2°C for a further 10 days. The mycosis of both isolates was recorded by means of noting the number of dead larvae, and by observing the characteristic coloration of the conidia on the agar plates (Fig. 3.4A, B).

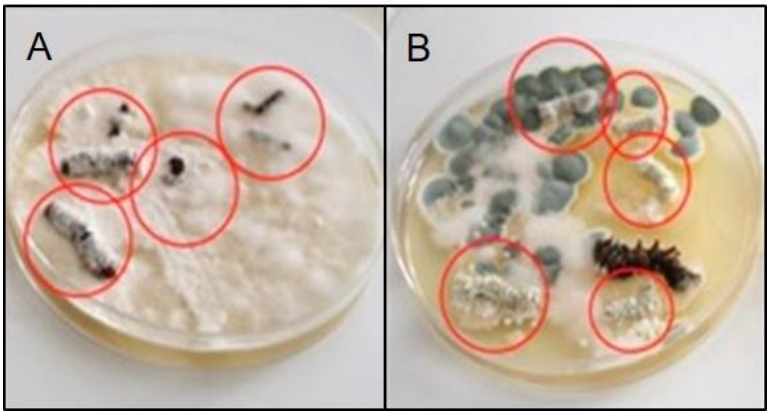

FIGURE 3.4

A: Screening of *Metarhizium anisopliae* against larvae of *Agoma trimenii* on Sabouraud Dextrose Agar (SDA) plates; B: Screening of *Beauveria bassiana* against larvae of *Agoma trimenii* on Sabouraud Dextrose Agar plates (with red circles indicating the infected larvae).

RESULTS

Pathogenicity of entomopathogenic nematodes

Pupae

In all the bioassays, zero mortality and infection by EPNs was obtained against the pupal stage of *A. trimenii*. The pupae treated with both EPN isolates eventually emerged as moths after ± 6 months, further proving that both of the isolates had failed to infect the pupae. No mortality was observed in the control group.

Larvae

In all the bioassays, both EPN species, *H. noenieputensis* and S. *yirgalemense*, caused 100% mortality after 48 h of exposure against the different larval instars of *A. trimenii* (ranging from 1.5 cm to 4.5 cm in length), at a concentration of 100 IJs/insect (Fig. 3.5A). The mean number of *H. noenieputensis* and *S. yirgalemense* IJs recovered per cadaver was 16 and 29, respectively (Fig. 3.5B). No control group replicates showed mortality.

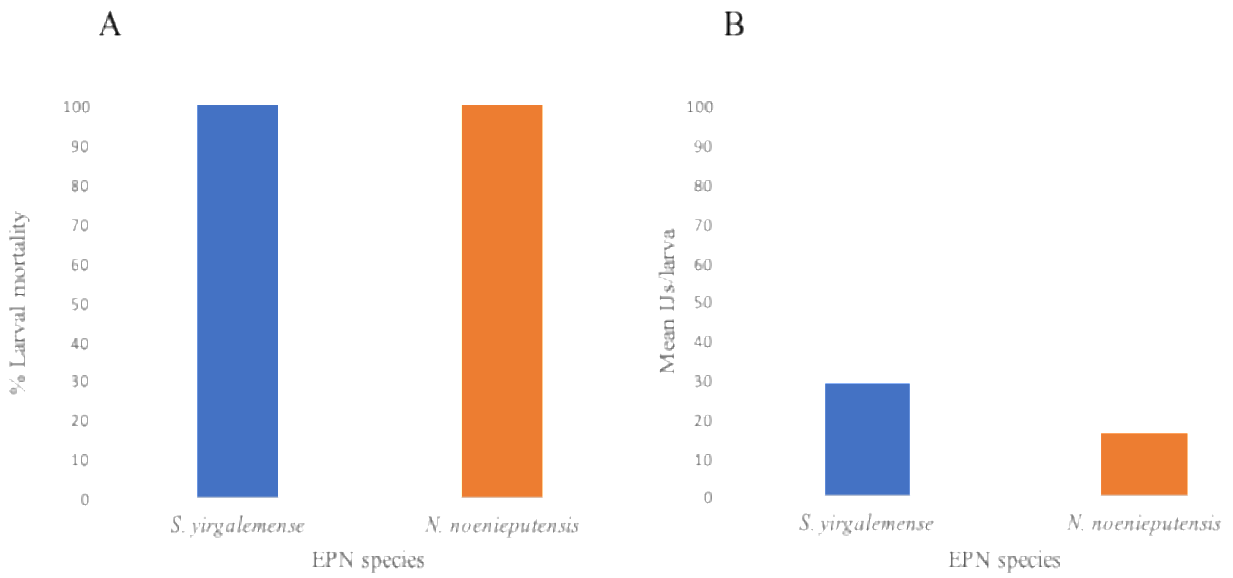

FIGURE 3.5

A: Percentage morality recorded for *Agoma trimenii* after exposure to *Steinernema yirgalemense* and *Heterorhabditis noenieputensis* at a concentration of 100 IJs/insect; B: Mean percentage of infective juveniles recovered from larval cadavers of *Agoma trimenii*, after exposure to *Steinernema yirgalemense* and *Heterorhabditis noenieputensis* at a concentration of 100 IJs/insect.

Pathogenicity of entomopathogenic fungi

Pupae

Similarly, screening of the EPF isolates, *B. bassiana* and *M. anisopliae*, against *A. trimenii* pupae showed zero mortality. Two pupae treated with *M. anisopliae* and one pupa treated with *B. bassiana* were assumed to be dead, as no movement was observed when they were held against the heated hotplate. However, upon inspection under the microscope, all three pupae concerned were found to have been damaged previously, possibly as a result of excavation in the field while the sampling and collecting was taking place. Therefore, 28 of the 30 pupae treated with *M. anisopliae* were confirmed to be alive after the set period, while 29 of the 30 pupae treated with *B. bassiana* were confirmed to be alive, after each individual was held against a heated hotplate so as to enable the observing of any signs of movement in response to the heat. The characteristic coloration of fungal spores for each isolate was found to be lacking when the SDA plates were examined for mycosis.

Larvae

In all the bioassays, the percentage viability of both the EPF isolates used was 100% at 10 days post treatment (Fig. 3.6). All larvae that were treated with *M. anisopliae* and B. *bassiana* died within 5 days of screening. After transfer to the SDA plates, at 6 days post treatment, the larvae treated with *M. anisopliae* showed varying shades of green fungal spores, which indicated mycosis. However, the agar plates with *B. bassiana*-treated larvae showed a red/yellow colouration that indicated possible bacterial infection. No mortality was observed in the control group.

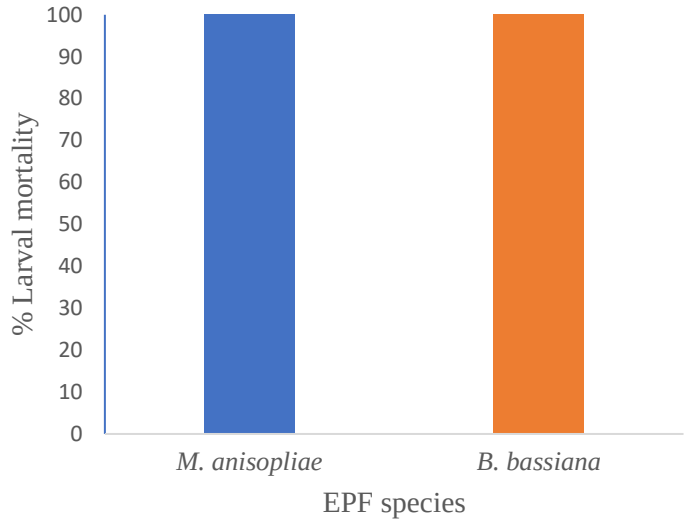

FIGURE 3.6

Percentage mortality recorded for *Agoma trimenii* larvae after exposure to *Metarhizium anisopliae* and *Beauveria bassiana* at a concentration of 0.2 ml/500 ml and 0.5 g/500 ml distilled water, respectively.

DISCUSSION

Good control potential against *A. trimenii* was exhibited by both the local EPN and the commercially available EPF isolates, when they were tested against the larval stage of *A. trimenii*. In contrast, the pupal stage showed no symptoms of infection from either the EPN or the EPF isolates.

Although both of the EPN isolates showed 100% mortality against the larval stage of *A. trimenii*, the mean number of IJs recovered from the larval cadavers infected with *S.*

yirgalemense was almost double that of those retrieved from the larval cadavers infected with *H. noenieputensis*. As shown by previous studies (Simoes & Rosa, 1996; Shapiro-Ilan *et al.*, 2002), the *Heterorhabditis* species was expected to prove more effective than was the steinernematid, as the former species have smaller maximum body diameters (± 528 µm) than do the latter, thus enabling them to have easier access through the natural openings of the host insect than do the latter (Malan *et al.*, 2014). In the current study, it appears that, regardless of its slightly bigger body size, *S. yirgalemense* (± 635 µm) (Nguyen *et al*., 2004) was the more successful candidate in the screening of EPNs against the larvae of *A. trimenii.* Therefore, its ability to infect and kill *A. trimenii* larvae was an important finding in the current study. The good potential of EPNs to control the soil life stages of numerous pests, including false codling moth, *Thaumatotibia leucotreta* (Malan *et al*., 2011), and the codling moth, *Cydia pomonella* (De Waal *et al*., 2011; Odendaal *et al*., 2016a, b), has resulted in research being undertaken into mass culturing (Ferreira *et al.*, 2016) and formulation. The pathogenicity of *S. yirgalemense* to *A. trimenii* larvae further emphasises its potential to control more than one target, which should enhance its acceptability by, and usefulness for, farmers. In future, the isolates should be tested in additional bioassays, focusing on factors influencing effectivity, and they should be evaluated under field conditions, so as to enable the investigation of their potential for incorporation into a biological IPM strategy.

The screening of both EPF isolates against the larvae of *A. trimenii* resulted in a high infection rate, as 100% mortality was observed in all the individuals treated. At 10 days post treatment, the SDA plates containing individuals treated with *M. anisopliae* showed the typical characteristic colouration of varying shades of green, indicating mycosis. Despite the lack of fungal growth of *B. bassiana* on the SDA plates, the larvae that died can still be concluded as having done so as a result of their infection, as each individual was declared dead after 5 days from the initial screening, compared to the control group, which showed no mortality. A study comparing the virulence among isolates of the *Metarhizium* and *Beauveria* species showed significant variations among the species and isolates screened against the greater wax moth, *Galleria mellonella* (Linnaeus) (Lepidoptera; pyralidae), and the meadow grasshopper, *Chorthippus parallelus* (Zetterstedt) (Orthoptera; Acrididae) (Anbesse *et al.*, 2008). The isolates of *M. anisopliae* were found to be more virulent than were those of *B. bassiana*, possibly as a result of the former having a wider host range than the latter (Ferron, 1978; Anbesse *et al*., 2008). To support the above-mentioned outcome, Ansari *et al*. (2004) found that the isolates of *M. anisopliae* were more virulent than were the isolates of *B. bassiana* and

other fungal isolates, in a study that selected a highly virulent fungal isolate from among 34 different EPF species.

Although both EPF isolates showed 100% mortality against the larval stages of *A. trimenii*, the pupal stage showed no susceptibility. The initial observations, of external sporulating fungal growth at the location of the conidial applications on the pupae, but no signs of infection/death, resemble the results that were obtained by Boucias & Latgé (1988) and Sitch & Jackson (1997). Coombes (2012) showed that fungal conidia germinated, and grew, on the surface of resistant insects, but that they were unable to penetrate and cause infection in the insects. The extraneous growth on resistant insects may reflect a lack of suitable stimuli for the initiation of penetration (Sitch & Jackson, 1997). The chemical stimuli (proteins, lipids and carbohydrates) might be key factors that determine whether or not insect species are susceptible to fungal attack. The pupal cuticle of *A. trimenii* provides the stage concerned with a higher level of protection from soil-dwelling fungi than that which is possessed by the larval stage. Anand *et al*. (2009) established that the pupae of *Spodoptera litura*, which resisted infection, took ± 2 to 5 days longer to emerge than did the pupae not exposed to the fungus. Additionally, Hafez *et al*. (1997) found that the pupae of the potato tuber moth, *Phthorimaea operculella*, which were successful in their emergence, despite exposure to *B. bassiana*, showed reduced fecundity. The indirect effects of EPF isolates on *A. trimenii* were, however, not the focus of the present study, but, rather, it was the ability of the EPF isolates to cause death at the soil-inhabiting pupal stage that was of primary concern. Despite the failure to kill the pupae involved, the findings obtained in the research provide future scope for testing the indirect effects of both *M. anisopliae* and *B. bassiana* on *A. trimenii* pupae. Control at the pupal stage is desired, as, if achieved, it would serve to eliminate the challenge set by the subsequent egg-laying by adult moths, and it could, therefore, potentially significantly reduce the size of subsequent *A. trimenii* populations. However, determining whether *A. trimenii* fecundity is reduced in the presence of the EPF isolates used in the present study should prove to be a worthwhile venture. By definition, biological control alone is unlikely to eliminate the problem completely, but it should, rather, tend to suppress and maintain the pest population levels below a predetermined economic threshold (Orr, 2009). However, if the level of fecundity is reduced, it can be assumed that the subsequent populations should also be smaller. Testing all the EPN and EPF isolates against the prepupal stage of *A. trimenii* should be the next step in the screening of bioassays. Infection can possibly occur during the short window period of the last larval instar that spends a short amount of time in the soil, prior to pupation and the length of

time taken for the newly developed moths to emerge from the soil. During the prepupal period, the cuticle tends to be softer and more malleable than is the heavily sclerotized cuticle of the fully formed pupa, thus making it more challenging for the IJs to penetrate and infect the host. According to many researchers, the stage of development of the insect has a significant effect on its susceptibility to EPNs (Kurtz *et al*., 2009; Ansari & Butt, 2012; Ma *et al*., 2013). Kaya & Hara (1980) showed that the prepupal stage of *G. mellonella, Spodoptera exigua* and *Mythimna unipuncta* was the most susceptible stage, exhibiting the highest mortality across all tested EPN concentrations. Developmental events during the pupal stage also appear to influence the IJ penetration rates (Dolinski *et al*., 2006). Additionally, Buitenhuis & Shipp (2005) suggest that differences in the susceptibility of the developmental stages of the western flower thrips, *Frankliniella occidentalis,* to EPNs stem from differences in their mobility. The non-feeding prepupae of *A. trimenii* are immobile, only responding via slight movement to mechanical stimuli. Therefore, the prepupae of *A. trimenii* can be argued as being the comparatively susceptible stage to EPNs.

Both the EPNs and the EPF investigated in the current study could be used cost-effectively in combination to control *A. trimenii* during the soil-inhabiting stage of the prepupae. The combinations might not only decrease the use of broad-spectrum chemical pesticides, but they might also provide seasonal control due to their ability to recycle under field conditions (Anbesse *et al*., 2008). Therefore, both the EPNs and the EPF can offer long-term control in the field. Such an approach is suitable for adoption in countries like South Africa, especially in the case of table grape producers who cannot afford to use chemicals close to harvest, because of the residues that might otherwise be left on the grapes. Studies have shown that the combined application of both biological and chemical insecticides results in a greater total effect than does the sum of their individual effects, suggesting that a combined application might offer a likely approach in terms of pest management concerning different agricultural systems (Stokwe, 2016). Barbercheck & Kaya (1991) investigated the interaction of *H. bacteriophora* and *B. bassiana* against the beet armyworm, *Spodoptera exigua* larvae, showing that combining the two agents resulted in greater host mortality compared to when they were tested separately. Therefore, in the case of EPNs that attack mainly soil-inhabiting pests, the adoption of combined applications with EPF might be a promising approach to the supplementing of control. Also, the secondary effect of *S. yirgalemense* on the key pests of table grapes, like false codling moth (Steyn, 2018) and the banded fruit weevil (Ferreira, 2010), which are highly susceptible to EPN, should prove to be a major added advantage of using EPNs.

CONCLUSION

The results obtained in the current chapter have provided useful information on whether EPNs and EPF can be used to control *A. trimenii*. From the findings made, it can be concluded that the tested EPN and EPF isolates are good potential biological control agents against the larval stages of *A. trimenii*. However, the isolates concerned cannot be used to control the pupal stages of *A. trimenii*, as zero mortality was observed across all screenings. Testing EPN and EPF isolates on the prepupal stage of *A. trimenii* holds potential for controlling the population at a time of zero damage to crops. As the results were determined under controlled laboratory conditions, whether the EPN and EPF isolates can perform as effectively under semi-field conditions remains to be tested. Future research should be directed at investigating the effect of the EPF isolates, available as commercial products, either individually, or in combination with EPNs as potential biological control agents, against *A. trimenii*. It should also consider the indirect effects of EPNs and EPF on the pupal stage of *A. trimenii*, so as to broaden the existing control options.

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Chapter 4

Insecticide Efficacy against Trimen's False Tiger Moth, *Agoma trimenii.*

ABSTRACT

Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae) has developed pest status in vineyards in the Northern Cape and Groblersdal areas of South Africa and control options are lacking. The aim of this study was to test the susceptibility of *A. trimenii* larvae to three commercial products, Delegate®WG, Steward®150 EC and three different doses of DiPel®DF under laboratory conditions. Semi-field trials were performed to test the potential of DiPel ® DF against *A. trimenii* larvae, applied at different water volumes (A: 50g/1000L/ha and B: 42g/1200L/ha) and to compare spray coverage between top and bottom leaves of vines. The residual activity of a DiPel®DF, when applied at different water volumes, was investigated daily and compared between top and bottom leaves over a 7-day period. Delegate®WG, Steward®150 EC and the recommended dose of DiPel®DF showed 100% larval mortality within 7 days. Delegate®WG and the recommended dose of DiPel®DF proved to be the fastest acting products. The recommended dose of DiPel®DF (0.25g/500 ml distilled water) proved the most effective dose (in comparison to halved and doubled dosages) and showed 100% mortality after 5 days of application. Increasing the water volume of a spray application of DiPel®DF showed no significant increase in larval mortality for combined top and bottom leaves. Generally, bottom leaves displayed higher larval mortality compared to top leaves when treated with either water volume. A reduction in insecticidal activity for DiPel®DF applied at both water volumes was seen between leaves picked 4 days after spraying and leaves picked 5 days after spraying, and no mortality was observed after day 6. Higher larval mortality was obtained on the picked bottom leaves throughout the 7-day period for both water volumes of DiPel®DF in comparison to top leaves, suggesting higher residual activity on bottom leaves of vines. Future research should be aimed at increasing spray coverage and residual activity of DiPel®DF as well as using all tested products within an integrated pest management system.

Key words: Delegate®WG, insecticide, Steward®150 EC, DiPel®DF, persistence; spray coverage

INTRODUCTION

Trimen's false tiger moth, *Agoma trimenii* Felder (Lepidoptera: Agaristidae), is an indigenous lepidopteran that is classified within the Noctuidae family and the subfamily Agaristidae. The moth recently developed pest status in parts of South Africa, after reports of moth infestations in vineyards in summer rainfall areas (Pretorius *et al*., 2012). *Agoma trimenii* is considered a severe agricultural pest, targeting the young shoots and leaves of wine, table and raisin grapes in the Northern Cape and Limpopo provinces of South Africa. As the economic importance of *A. trimenii* in the grape-producing regions of South Africa increases, so does the need to control its increasing population.

At present, the only available measure of control is repeated application of chemical insecticides. However, such insecticides, for use against *A. trimenii* are currently still undergoing registration. Despite the suppression of pest populations with insecticides, undesirable effects are often unavoidable (Gullan & Cranston, 2014.) This could result in the development of secondary pest outbreaks that are currently under natural control (Pretorius *et al*., 2012). Concerns over human health and environmental impacts have encouraged the development and utilisation of alternate pest management programmes, products and technologies (Gullan & Cranston, 2014). Integrated pest management (IPM) strategies address such concerns and accommodate new pest control techniques.

Potential biological control strategies for *A. trimenii* include the application of formulated biological derived insecticides, such as Delegate®WG (containing the active ingredient, spinetoram, which is derived from the fermentation of *Saccharopolyspora spinosa* Mertz & Yao (Actinomycetales: Pseudonocardiaceae), a naturally occurring soil bacterium) and DiPel®DF (a *Bt*-based insecticide formulated from *Bacillus thuringiensis* var. *kurstaki* (Bacillales: Bacillaceae), that are specifically pathogenic to the larval stages of Lepidoptera). Such biological insecticidal derivatives, exhibit many beneficial traits, including offering minimal risk to human health, the absence of toxic residues in crops, minimal risk to beneficial and other non-target insects, and host specificity (Inglis *et al*., 2001; Goettel *et al*., 2005). Testing the above-mentioned biologically derived insecticidal options on *A. trimenii* is important, as no such control options have previously been evaluated.

Delegate®WG boasts a broad insecticidal spectrum, especially against all growth stages of lepidopteran pests including codling moth, *Cydia pomonella*, light brown apple moth, *Epiphyas*

postvittana (Walker) (Lepidoptera; Euathropoda) and oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) (Dripps *et al*., 2008; Magalhaes & Walgenbach, 2011; Sial *et al*., 2011). Its mode of action may be either directly through contact with the body surface (contact toxicity), or through ingestion toxicity (Shimkawatoko *et al*., 2012). Due to its unique mode of action, Delegate®WG could be a rotational product for use in an IPM programme (Bacci *et al*., 2016).

The insect growth regulator, Steward®150 EC, can be classified as an insecticide that causes a disruption to crucial physiological functions associated with the life cycle of insect development and metamorphosis (Sanchez-Bayo, 2012). Steward®150 EC is administered directly by contact through the body surface or indirectly through ingestion (Wing *et al*., 2000). Its novel mode of action of inhibiting sodium entry into nerve cells, results in paralysis (inhibited feeding) and death of the target pest within three to five days (Dinter & Wiles, 2000). With its unique mode of action and its minimal impact on beneficial parasitoid and predatory insects, it has great potential as a component of an IPM programme (Nowak *et al*., 2001).

DiPel®DF is characterized by numerous crucial attributes that favour its application in a pest management programme, namely its nontoxic nature to both plants and vertebrates, as well as its relatively specific action on target insect species (Roditakis, 1986). Mortality of infected insects usually occurs within 2-3 days as a result of the effects of septicemia, or can occur directly (Waites *et al.*, 2009). DiPel®DF has proven to be a successful biological control agent against two Lepidopteran pest species present in South Africa: the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), which is highly resistant to conventional insecticides, as well as the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (Tabashnik *et al*., 1990; Devetak *et al*., 2010). DiPel®DF has is specifically used for the control of lepidopteran larvae, therefore there is scope to test this product on *Agoma trimenii.*

The use of the two environmentally-friendly biologically derived pesticides, as well as a ‘softer’ pure chemical product for the control of *A. trimenii*, requires knowledge of their susceptibility, prior to application on a large scale. Bioassays are the starting point for any control investigation in which virulence is of importance, as they allow for the removal of factors that could reduce virulence towards the target host (Coombes, 2012). This study investigated the potential of less harsh insecticides such as Delegate®WG and Steward®150 EC and DiPel®DF to control *A. trimenii* under laboratory conditions and determined their feasibility for use as part of an IPM program directed at the management of the pest. Laboratory

bioassays were performed against the larval stages of *A. trimenii*, to determine the potential of each pesticide as a control agent against this stage of the insect. Furthermore, semi-field trials in table grape vineyards were performed to test the potential of DiPel®DF against *A. trimenii* larvae, applied at different water volumes, spray coverage, as well as the residual activity of DiPel®DF after application and ingestion by larvae.

MATERIALS AND METHODS

Source of larvae

Larvae of *A. trimenii* were collected from the untreated demarcated field site on Farm A in the Northern Cape province, South Africa. Various larval instars were collected in November 2017 and February 2018. Larvae ranged from 1.5-4.5 cm in length, encompassing different larval instar stages. The larvae were stored in plastic 2 L containers, provided with a mesh-covered hole in the lid to ensure adequate ventilation. The containers were stored in conditions similar to that of their natural environment.

Source of Delegate®WG, Steward®150 EC and DiPel®DF

The commercial product Delegate®WG (Dow AgroSciences), Steward®150 EC (Du Pont) and DiPel®DF (Philagro) was obtained from South African distributers and applied at the recommended dose for lepidopteran larvae, as prescribed on the product label. Additionally, DiPel®DF was applied at half the recommended dose and double the recommended against *A. trimenii*.

Field sites

Semi-field trials were carried out on a commercial table grape farm in the Northern Cape province of South Africa, with high infestation levels of *A. trimenii*, during the growing season of the table grapes, over a 7-day period in November 2017 and February 2018. Semi-field trials were conducted on two field sites (A and B), each site comprised of a netted block of the table grape, Thompson Seedless. The vines followed a double-gable trellising system, with plant spacing of 1.8 m, and row width of 3.3 m. For both field sites, four rows of 20 vines were demarcated and left untreated by pesticides.

Laboratory bioassay protocols

Two replicate laboratory bioassay trials with Delegate®WG, Steward®150 EC and DiPel®DF against the larval stages of *A. trimenii* were conducted during November 2017 and February 2018. Fresh, untreated vine leaves still attached to their stems, were lightly coated by spraying the recommended concentration of Delegate®WG (0.05 g/500 ml) and Steward®150 EC (0.2 ml/ 500 ml), and three different doses of DiPel®DF (Treatment 1 (TMT1): half the recommended dosage = 0.125 g/500 ml, Treatment 2 (TMT2): recommended dosage = 0.25 g/500 ml and Treatment 3 (TMT3): double recommended dosage = 0.5 g/500 ml in distilled water). The leaves were left to dry for ± 20 min and were placed into 2 L plastic containers according to the product tested and lined with paper towels. For both replicates per treatment, larvae (n = 12 per container) were added to the respective containers, ventilated with a mesh-covered hole in the lid. An identical control (n = 12 larvae) per treatment was prepared on the day of screening. Leaves were sprayed with distilled water and dried, before being placed into 2 L plastic containers with larvae. All plastic containers were kept outside under natural conditions of semi-shade. Treatment and control larvae were left to feed on the treated leaves. Effectivity of control was assessed after application by recording mortality every day for a period of 7 days and inspecting each larva for movement and change in physical appearance.

Field application and efficacy assessment of DiPel®DF

Replicate field trials of the insecticide, DiPel®DF, were performed in November 2017 and February 2018 at two untreated sites on Farm A. DiPel®DF was applied in the field with the same sprayer at the recommended concentration, 50 g/ha, as two separate treatments of different water volumes (Treatment A: 50g/1000 L/ha and Treatment B: 42g /1200 L/ha), i.e., increasing the water volume of the constant concentration of DiPel®DF. Trials commenced in the early morning (07h00), to avoid too high levels of UV radiation. With the use of a cima centrifugal sprayer, treatments were applied to the leaves of vines both sites, on each of the four rows of vines at a speed of 3.6 km/h (Fig. 4.1).

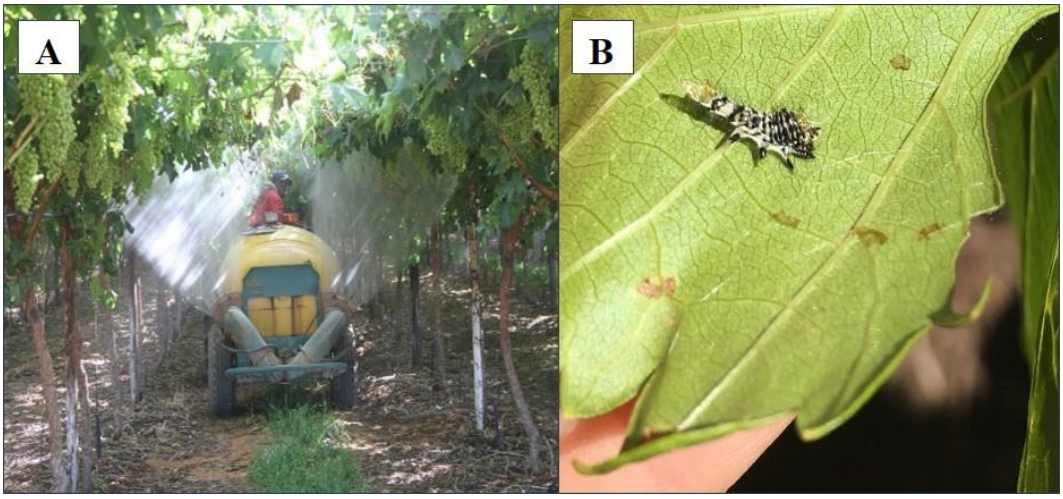

FIGURE 4.1

A: Cima sprayer, spraying DiPel®DF at two water volumes (A: 52g/1000l/ha and B: 40g/1200l/ha) at the field site; B: Larva of *Agoma trimenii* after approximately 7 days after application of DiPel®DF.

After application, leaves from the top and bottom of the vines from the middle row of both treatment sites were collected every day, at the same time (08h00), for 7 days. The top and bottom leaves, for each day, were kept separate in two plastic 2 L containers, lined with paper towels. Fresh field-collected larvae (n =12) were then added to each container of both treatment replicates and left to feed on the treated leaves. An identical control (n = 12 larvae) was prepared on each day with leaves picked from untreated vines and sprayed with distilled water. Mesh-covered holes in the lids of the containers ensured adequate ventilation. All treatments and control were kept outside under natural conditions. Each collection of leaves, picked daily for 7 days, received fresh larvae (n = 12) on the day of collection and larval mortality was recorded per container after 24 h of feeding for a period of 7 days, i.e., the first collection of leaves with added larvae, had 7 days of recorded observations, whereas the last day's collection of leaves with added larvae, had only one observation after the final 24 h feeding period.

Larval mortality was used to compare effectiveness of the two water volumes, as well as the spray coverage between top and bottom leaves for each water volume application. Residual activity (activity of the product on leaves so many days after application) was presented by displaying the decrease in larval mortality for the two water volumes of DiPel®DF over time. Larval mortality was recorded after 24 h of feeding on the treated leaves for each day after spraying and will be presented.

Statistical Analyses

Abbott's formula (Abbott, 1925) was used to correct larval mortality of *A. trimenii* larvae for both replicate trials, in order to compensate for natural deaths. All statistical analyses were done using STATISTICA version 13. Repeated measures ANOVA (analysis of variance) was conducted to analyse mortality over time for each bioassay of Delegate®WG, Steward®150 EC and DiPel®DF. A post-hoc comparison of means was done by using the Fisher LSD test. All statistical analyses were done using STATISTICA version 13. A two-way ANOVA was conducted to analyse larval mortality as a result of the two water volumes of DiPel®DF, and between top and bottom leaves. A one-way ANOVA was conducted to compare the means of larval mortality of the two water volumes, as well as the means of larval mortality from top and bottom leaves. To confirm activity of DiPel®DF on picked leaves over the 7-day period, repeated measures ANOVA was conducted to compare larval mortality between consecutive days from which leaves were sprayed. A three-way ANOVA was used to compare larval mortality between picked top and bottom leaves for each water volume over the 7-day period after spraying and a post-hoc comparison of means was done by using the Fisher LSD test.

RESULTS

Pathogenicity of Delegate®WG, Steward®150 EC and DiPel®DF against larvae

In the treated groups, mortality increased significantly over the 7-day period for Delegate®WG ($F_{(6,6)} = 19.400$; $p < 0.05$) and Steward®150 EC ($F_{(6,6)} = 6.512$; $p < 0.05$) with 100% mortality after 7 days (Fig. 4.2 and Fig. 4.3). Larval mortality due to Delegate®WG increased at a faster rate than did larval mortality due to Steward®150 EC. By day 4 of the bioassays, larval mortality after treatment with Delegate®WG was 91.67 ± 9%, and reached 100% by day 5, compared to Steward®150 EC which resulted in 66.67 ± 20% by day 4 and reached 100% by day 6. Mortality due to Delegate®WG, revealed that a significant increase in larval mortality occurred between the first and second day after ingestion ($p = 0.0396$), in comparison to other consecutive days (Fig. 4.2). Data showed no significant difference between consecutive day comparisons for mortality due to Steward®150 EC ($F_{(6,6)} = 6.512$; $p > 0.05$).

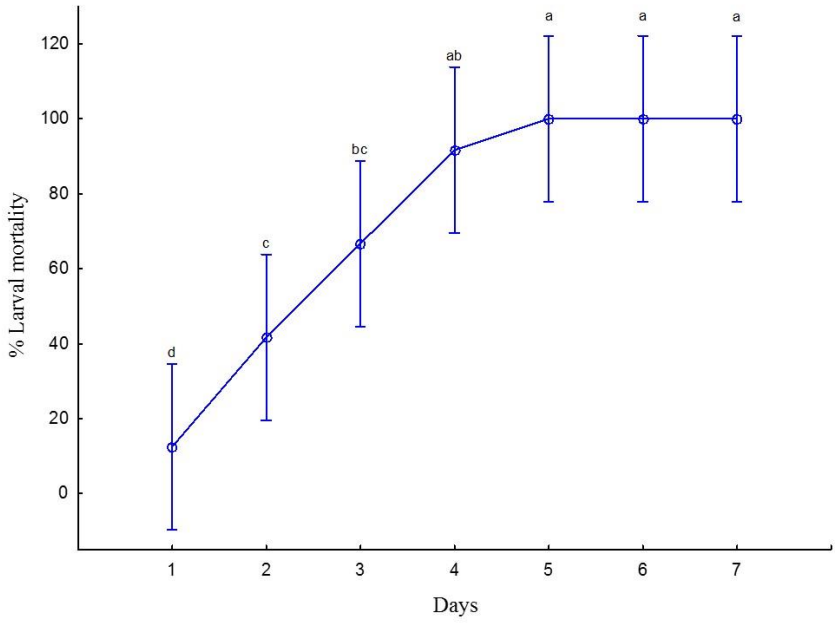

FIGURE 4.2

Mean percentage mortality (95% confidence interval) of *Agoma trimenii* larvae after ingestion of leaves sprayed with Delegate®WG at a concentration of 0.2 ml/500 ml distilled water, over 7 days (one-way ANOVA; $F_{(6,6)} = 19.400$; $p < 0.05$). Bars sharing a common letter are not significantly different.

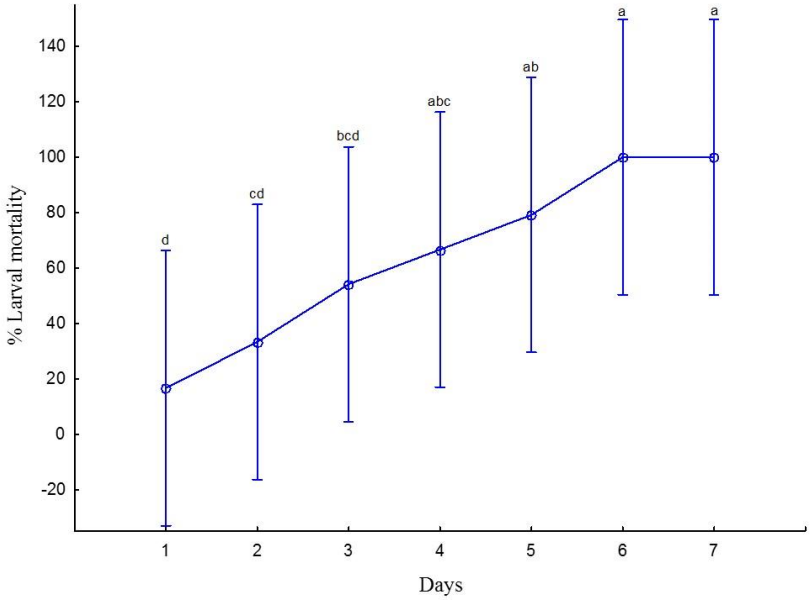

FIGURE 4.3

Mean percentage mortality (95% confidence interval) of *Agoma trimenii* larvae after ingestion of leaves sprayed with Steward®150 EC at a concentration of 0.05g/500 ml distilled water, over 7 days (one-way ANOVA; $F_{(6,6)} = 6.512$; $p < 0.05$). Bars sharing a common letter are not significantly different ($p < 0.05$).

In laboratory bioassay involving the three different doses of DiPel®DF, control groups for all three treatment bioassays showed no mortality over the 7-day period. Larval mortality due to the three doses of DiPel®DF over the 7-day period is presented in Fig. 4.4. By day 4 of the bioassay, mortality after applying the recommended dose (TMT2) was 83.3 ± 12% and reached 100% by day 5, compared to the half recommended dose (TMT1), which resulted in 75 ± 12% by day 4, and remained unchanged, and the double recommended dose (TMT3), which resulted in 95.8 ± 12% by day 3 and 100% by day 4. Data analysed after the 7-day period showed no significant difference in total larval mortality between the means of each treatment ($F_{(12,18)}$= 0.84324; $p > 0.05$). However, a pairwise comparison of larval mortality across all three treatments per day using the Fisher LSD (minimum mean difference = 41.67), showed a significant difference between TMT1 and TMT3 on the third day of the bioassay trial (p = 0.025).

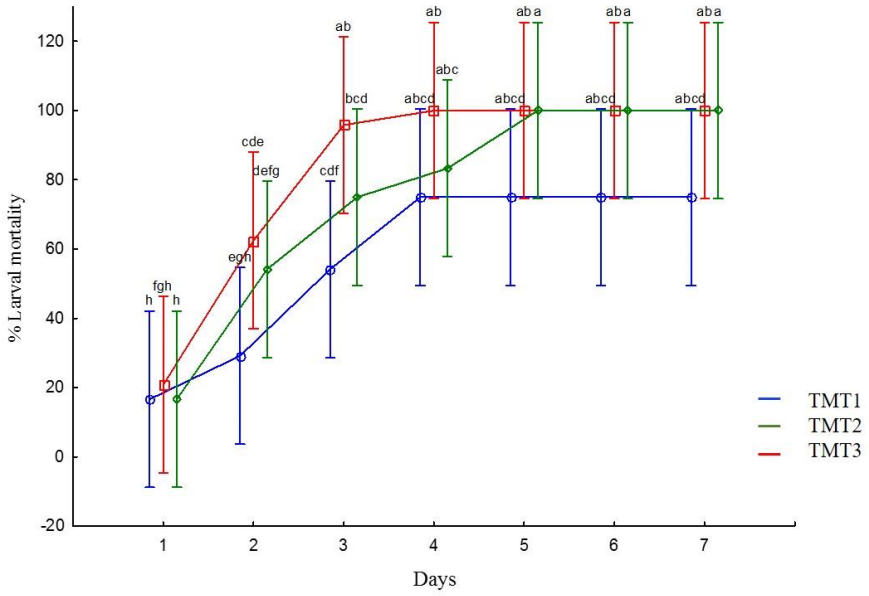

FIGURE 4.4

Mean percentage mortality (95% confidence interval) of *Agoma trimenii* larvae after ingestion of DiPel®DF: (TMT1: 0.125 g/500 ml, TMT2: 0.25 g/500 ml, TMT3: 0.5 g/500 ml in distilled water) over 7 days (repeated measures ANOVA; $F_{(12,18)}$ = 0.84324; $p > 0.05$). Bars sharing a common letter are not significantly different ($p < 0.05$).

All tested insecticides resulted in 100% larval mortality within the 7-day test periods. Delegate®WG and the recommended dose of DiPel®DF achieved 100% larval mortality by day 5, while Steward®150 EC required one more day, and when the recommended dose of DiPel®DF was doubled, 100% mortality was achieved by day 4.

Field trials

Effectivity of DiPel ® DF applied at different water volumes and subsequent spray coverage

Total larval mortality (n = 168) was compared between the two water volume applications of DiPel®DF for the 7-day period, as well as larval mortality between the top and bottom leaves (Fig. 4.6). Water volume B (42g/1200L/ha) resulted in higher larval mortality for combined top and bottom leaves (55 ± 5%), compared to larval mortality of water volume A (50g/1000L/ha) for both top and bottom leaves (51 ± 5%), but not significantly so ($F_{(1,4)}$ = 3.1075, $p > 0.05$). For both water volumes A and B, higher larval mortality was seen on leaves picked from the bottom of the vine (27.35 ± 4% and 30.12 ± 4% respectively), compared to the top leaves (23.8 ± 4% and 24.96 ± 4% respectively). This was significantly higher for water volume B (p = 0.037), but not statistically significant for water volume A.

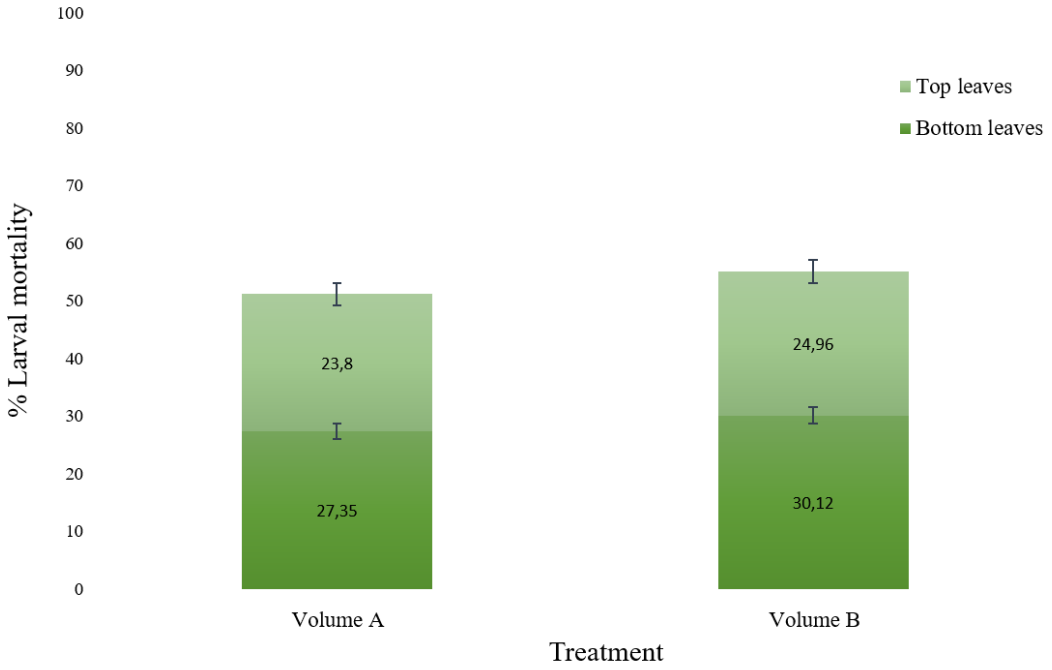

FIGURE 4.5

Mean percentage mortality of *Agoma trimenii* larvae after ingestion of DiPel®DF applied at two water volumes (A: 50 g/1000 L/ha and B: 42 g/1200 L/ha), between top and bottom leaves of vines after 7 days.

Residual activity of DiPel®DF applied at different water volumes and subsequent spray coverage

Residual activity of DiPel®DF applied at both water volumes, A and B, is presented by displaying the decrease in larval mortality after 24 h of feeding on picked top and bottom leaves, from 1 to 7 days after the leaves were sprayed (Fig. 4.6). For both water volumes, larval mortality was higher on picked bottom leaves, compared to top leaves. Larval mortality (n = 81) due to water volume B was higher than that of water volume A (n = 67), although not significantly so. For both water volumes A and B, it appeared that larval mortality showed the greatest decrease from days 4 (38 ± 5% and 59 ± 5% respectively) to 5 (21 ± 5% and 25 ± 5% respectively) after spraying, for combined top and bottom leaves and that by day 6, no larval mortality was recorded.

For water volume B, a pairwise comparison of larval mortality between consecutive days using the Fisher LSD (minimum mean difference = 37.50), revealed that mortality significantly decreased on picked bottom leaves 4 to 5 days after spraying (p = 0.0054), however for water volume A, no significant differences were established between consecutive days for picked top leaves or picked bottom leaves.

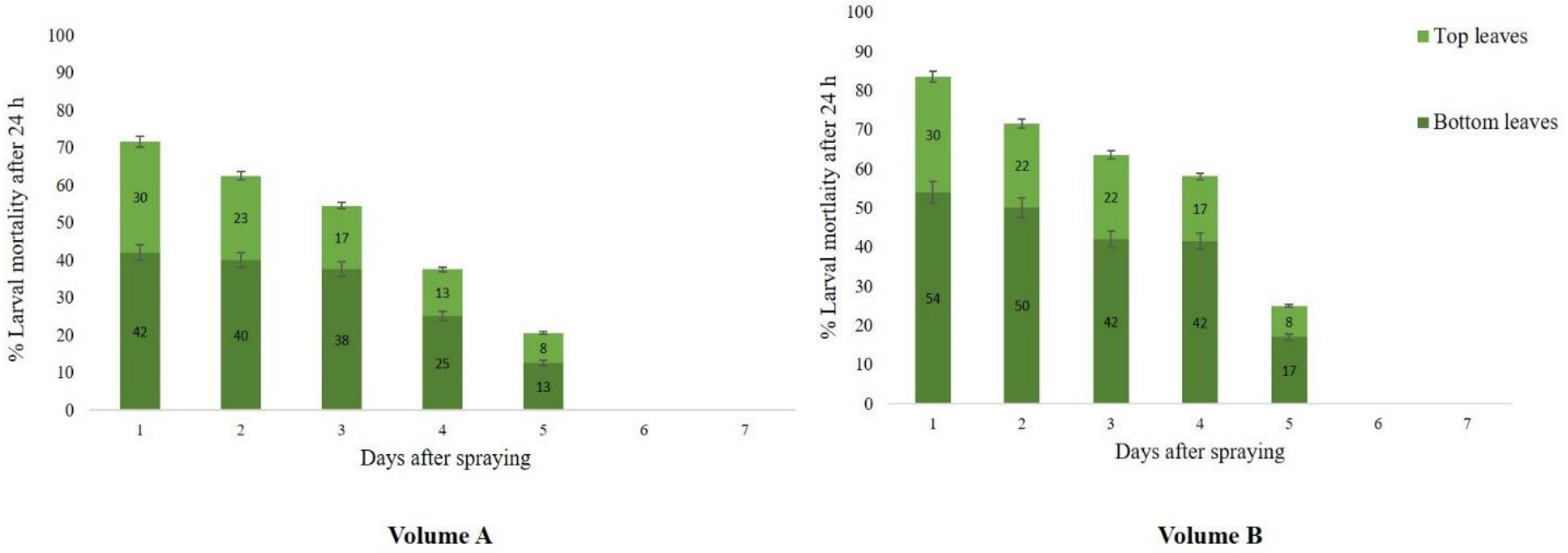

FIGURE 4.6

Larval mortality of *Agoma trimenii*, after 24 h of feeding on picked top and bottom leaves sprayed with DiPel®DF at two water volumes (A: 50 g/1000L/ha and B: 42 g/1200L/ha), over the 7-day period after the leaves were sprayed.

DISCUSSION

Results from laboratory bioassays showed that both Delegate®WG and Steward®150 EC had excellent activity against the early to mid-larval stages of *A. trimenii*. The knockdown effect of Delegate®WG on *A. trimenii* larvae was faster than that of Steward®150 EC. In both laboratory bioassays, infected larvae stopped feeding on leaves, displayed paralysis and developed curved and discoloured bodies. Infected larvae also expelled a green liquid from the mouth. Other infected larvae stopped feeding and remained alive for several days before they died. The results from these bioassays are in line with a study conducted by Abbas *et al*. (2015), who reported that in semi-field trials, Delegate®WG showed relatively better persistence and contact mortality of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), compared to Steward®150 EC, although the difference in larval mortality between the products was statistically non-significant. Dobie (2010), reported Delegate®WG to be highly efficacious against potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; Celechiidae), due to its quick knockdown ability, however Steward®150 EC provided longer residual activity. Liu *et al*. (2003), also reported on the longevity of Steward®150 EC, where one application of Steward®150 EC against the diamondback moth, *P. xylostella*, supressed larvae below an economic threshold for 14-21 days in the field. This slow acting property may, however, affect the behaviour and physiology of target insects persistently at different levels, yet failing to cause immediate death (Wang *et al*., 2011). Therefore, a trade-off exists between the fast action of Delegate®WG and the longer persistence of activity of Steward®150 EC. Nevertheless, both control options show excellent activity against larvae of *A. trimenii* and hold potential as exceptional rotational products for use in an IPM programme.

Laboratory bioassays testing the effectivity of different doses of DiPel®DF showed that the recommended dose (TMT2) and double the recommended dose (TMT3) give good control against larval stages of *A. trimenii*. Larval mortality as a result of TMT3, increased significantly on the third day after ingestion, compared to TMT2, which suggests that TMT3 caused a faster rate of mortality. However, this observation is only applicable to one day (day 3) and no significant differences in larval mortality between TMT2 and TMT3. It can be concluded that the significant difference in larval mortality on the third day of ingestion had no significant effect on the rate of larval mortality between TM2 and TMT3 over the 7-day period. This was confirmed, as no significant difference was observed between the means of each treatment after 7 days. TMT2 successfully controlled 95% of *A. trimenii* larvae after 3 days and effectively

killed 100% of larvae after 7 days, however larval mortality was low (< 23%) one day after treatment. This finding is supported by Rowell (2005), who observed that *B. thuringiensis var. kurstaki* did not work like most conventional insecticides, in that it had no contact or knockdown effect (larvae were not killed instantly after application). The observation from the laboratory bioassay, that larval mortality increased rapidly after 3 days of ingestion, is in accord with the product label recommendations of DiPel®DF, which states that after ingesting a lethal dose, larvae stop feeding within the hour and will die within several hours to 3 days. Not only did TMT2 result in 100% larval mortality, but it is also more cost-effective then Treatment 3 as less amount of product is required to result in 100% larval mortality. Therefore, TMT2 (the recommended dose of DiPel®DF) should be used to control *A. trimenii* larvae. It would be beneficial to apply this bioassay under semi-field conditions, as environmental factors, such as temperature and exposure to ultraviolet radiation (UV), play a significant role in the effectivity and longevity of the product (Abd, 2008).

Semi-field trials demonstrated the influence of water volume on the ability of a vineyard cima sprayer to effectively cover top and bottom leaves of vine canopies with DiPel®DF (Ebert *et al*., 1999). In field application, concerns such as coverage and application volume, function through their effect on pesticide deposit structure (Ebert *et al*., 1999). Results showed that larval mortality was significantly higher on the bottom leaves when volume B (42g/1200L/ha) was applied. This could be attributed to a greater spray coverage on bottom leaves. This finding is supported Brink *et al*. (2016), which observed that at higher spray volumes, lower leaf surfaces resulted in higher deposition quantity compared to upper leaf surfaces. Wise *et al*. (2010) reported that the volume of water used to carry the pesticide to the target, is one key factor of sprayer operation that can be varied by the grower to improve the level of coverage of the targeted crop. Therefore, adjusting application volume not only influences coverage, but also affects the concentration of active ingredient per deposit given a constant application rate (Ebert *et al*., 1999). Relatively little is known about how variation in water volume affects target coverage in grapevine canopies and even less is known about how this coverage affects the control of key vineyard pests with various pesticides (Wise *et al*., 2010). However, generally larger volumes result in greater coverage per plant (Ebert *et al*., 1999). Additionally, it is suggested that spray coverage may be improved by increasing spray volume and decreasing tractor speed (Sial & Brunner, 2010; Cavalieri *et al*., 2015).

Results from semi-field trials are expected to drastically differ in the real world under field conditions. *Agoma trimenii* larvae appeared to feed on leaves high up in the vine canopy, as less damage was observed on bottom leaves during the visual scouting and observational studies conducted in Chapter 2. Therefore, coverage is a challenge due to the high positioning of the leaves and the need to spray the adaxial side of these leaves, as the most damaging stage of *A. trimenii* (early to mid-level instars) occurs at these sites. In the semi-field trials, larvae had no choice but to feed on bottom leaves which may have resulted in higher larval mortality than would have occurred in the field under natural conditions.

Laboratory bioassays provide information on the relative toxicity of an insecticide, but it is the longevity of residual actives that informs growers about retreatment intervals. Prolonging the lifetimes of spray droplets on leaves increases the absorption and uptake of active ingredients (Knoche *et al*., 2000). Therefore, information on the evaporation time can help pesticide formulators to develop better products that can maximise uptake by leaves. It is of importance to establish the residual activity of DiPel®DF against the larvae of *A. trimenii* to incorporate appropriately timed spray applications into a control programme.

In this study, the loss of insecticidal activity over time was indicated by the decrease in larval mortality and an increase in the number of live larvae recorded. Results of semi-field trials showed a reduction in insecticidal activity for DiPel®DF applied at both water volumes, between leaves picked 4 days after spraying and leaves picked 5 days after spraying. This observation was similar to the product label recommendations of DiPel®DF, which states that after ingesting a lethal dose, larvae stop feeding within the hour and will die within several hours to 3 days, i.e. that after 3 days of ingestion, insecticidal activity will begin to decrease. This was observed for both water volumes, when larvae stopped feeding within hours of ingestion and mortality began after ± 24 hrs. Similar results were obtained in Copping & Menn (2000), who reported that feeding ceases within 2-48 h of ingestion of *B. thuringiensis*. It can be confirmed from results of this study that residual activity of the *Bt* toxins in DiPel®DF is prevalent for at least 3 days after spraying, regardless of an increase in water volume of applications. Therefore, water volume had no effect on the longevity of DiPel®DF actives. The lack of effect of spray volume on longevity of *B. thuringiensis* was also illustrated by Ali & Young, 1993, who tested the effects of rate and total spray volume on activity of *B. thuringiensis var. kurstaki* against the tobacco budworm, *Helopthis virescens* (Lepidoptera:

Noctuidae), in cotton terminals. It is of interest that this species and *A. trimenii* belong to the same family.

After ingestion of DiPel®DF treated leaves, larvae consumed little surface tissue before dying. This highlights the dual action by which larvae are killed; inhibited feeding which leads to starvation and direct rapid action of the endotoxin (δ-endotoxins) (Copping & Menn, 2000; Bravo *et al*., 2007). Inhibited feeding may suggest that contamination of mouthparts by *Bt* crystals, may be sufficient to kill those individuals which carry some crystals from the leaf surface, either by chance or by initial feeding activity (Bailey *et al*., 1996). From results of DiPel®DF laboratory bioassays, it was expected to observe nearly 100% larval mortality on leaves picked on the first day in the semi-field trials. However, this was not the case, as larval mortality was lower than expected for both water volumes. The low levels of larval mortality for both water volumes can be attributed to several factors. The residual activity of DiPel®DF is dependent on its persistence and breakdown under field conditions (Duffield & Jordan., 2000). Such conditions include ultraviolet radiation (UV) exposure in the field, which adversely affects the insecticidal activity of *B. thuringiensis* (Van Es & Tautmann, 1990). Light energy destroys spore viability, degrades the toxic protein and reduces insecticidal activity (Navon 2000). Additionally, as temperature increases, the rates of microbial and chemical reactions increase, so pesticide degradation occurs at a faster rate as the soil and air become warmer (van Es & Trautmann, 1990). Semi-field trials commenced at 07:00 during the summer period, when sunrise was recorded at ± 05:30. Had the spray application commenced before sunrise, temperature and UV radiation would be significantly lower in comparison to post sunrise. Significant improvements are possible to extend the residual insecticidal activity of DiPel®DF, by the discovery of formulations that protect *B. thuringiensis* from degradation by sunlight (Behle *et al*., 1997). For the purposes of this study, such improvements may prove beneficial in enhancing the residual activity of DiPel®DF on top leaves of the vine canopy. Higher larval mortality on picked bottom leaves for both water volumes over the 7 day period, as well as the significantly higher larval mortality on picked bottom leaves for volume B after day 4 of spraying, suggests a longer residual activity on the bottom treated leaves, regardless of water volume, which may be less effective for controlling *A. trimenii* populations, as the most damaging stages (early to mid-instars) feed on the highest leaves of the vine canopies which are exposed to significant UV radiation and subsequent high temperatures. Therefore, measures to enhance residual longevity of DiPel®DF are crucial for controlling the most aggressive larval stages of *A. trimenii*.

A long residual activity of pesticides can be a useful tool for pest management, but it is also a risk for resistance development in multivoltine species, due to exposure to low-residue levels (Pavan *et al*., 2014). Insecticides that employ a long-lasting effect against *A. trimenii* may have a selective pressure on the next generations (Pavan *et al*., 2014). Additionally, the high reproductive ability of *A. trimenii* and its aggressive larval foliar damage make the evolution of pesticide resistance a serious threat to the sustainability of the chemical control of this pest. The use of new pesticides for the control of *A. trimenii* requires knowledge of its current susceptibility under laboratory conditions prior to application at a large scale. Knowledge of susceptibility could prevent the rapid development of pest resistance, as was observed for banded leaf roller, *Choritoneura rosaceana* Harris (Lepidoptera: Tortricidae), to spinosad insecticide within 6 years of its introduction into the field (Dunley *et al*., 2006). Such knowledge provides the foundation for implementing a practical insect resistance management program.

The timing of application of pesticides as a means of pest control is becoming an increasingly more important issue. Insect phenology models such as those based on the work of Riedl *et al* (1976) for the codling moth, have been used to forecast population events such as adult emergence, egg laying and egg hatch. This information, together with knowledge of the toxicity of insecticides to different life stages of *A. trimenii* may be valuable in determining the appropriate timing of insecticide applications. The effectiveness of Bt application depends heavily on its timing. It is advised to spray early in the season, before high field populations of potential parasitoids and predators on the pest have been reduced by chemical pesticides and spraying after sunset instead of in the morning which can increase the persistence of the product in warm countries, where activity of the microbe persists for only 2-3 days (Navon, 2000). Under heavy pest population pressure, higher label rates should be applied, spraying intervals should be shortened and spray volume could be increased to improve spray coverage (Navon, 2000). Appropriate timing of spray application is crucial for *A. trimenii*, as this ensures a proper control method to be carried out and avoids overuse of DiPel®DF which can increase costs. Knowledge of life stage specificity is important for determining application timing in the field and for devising bioassays in resistance monitoring programmes (Magalhaes & Welgenbach, 2011). One of the most significant economic aspects of pest management using DiPel®DF, is the application against young larvae, preferably neonates, as it has been confirmed in laboratory and field bioassays that third instar larvae of Lepidoptera are less susceptible to the Bt products compared to younger larvae (Tan *et al*., 2008). Bt based biological pesticides are usually

applied when early instar larvae are present, as Bt susceptibility of mature larvae is very low (Navon, 2000) and older larvae are more tolerant (Sanahuja *et al*., 2011). This was confirmed in a laboratory experiment (Tan *et al*., 2008), on the bag worm, *Metisa plana* (Walker) (Lepidoptera: Psychidae), which showed that third instar larvae were more susceptible to *B. thuringiensis* than fifth instar larvae, and that the rate of mortality was faster for younger larvae. Variations in susceptibility to *B. thuringiensis* during larval development would be of value in establishing the best management strategies in terms of the timing of DiPel®DF application for *A. trimenii*.

Future studies can be performed in the laboratory and under field conditions to better understand the efficacy of the control options investigated in this study. All of the pesticides are sufficiently persistent to cause mortality for at least 7 days or longer. Studies that focus on mortality after 24 hours are important in determining the actual length of time that bioactivity will persist in the field. Furthermore, since many compounds cause the pest to stop feeding and thereby prevent damage, studies that evaluate the feeding time and damage after application are important to determine the level of control that can be expected.

Results obtained in the current chapter have provided useful information on whether Delegate®WG, Steward®150 EC and DiPel®DF can be used to control *A. trimenii*. From the findings, both Delegate®WG and Steward ® 150 EC prove effective against the larval stages of *A. trimenii*, however a trade-off exists between the fast action of Delegate®WG and the longer persistence of activity of Steward®150 EC. Results from laboratory bioassays showed that the recommended dose of DiPel®DF, according to the product label, is the most appropriate and cost effective dose for the control of *A. trimenii* larvae compared to halving or doubling the recommended dose. Results from semi-field trials suggested that increasing the water volume of a spray application of DiPel®DF will result in a greater spray coverage and subsequent larval mortality, highlighting the importance of achieving maximum spray coverage. Efforts to increase the residual longevity of DiPel®DF are imperative for effective application on top leaves of the vine canopy, as the most damaging stages of *A. trimenii* are located at these sites. It is suggested that knowledge of pest resistance, susceptibility of different life stages to all tested products and the subsequent appropriate timing of spray application, will prove advantageous in developing control programmes. Future research should be directed at investigating the effects of all products against *A. trimenii* under field

conditions, either simultaneously or in rotation as part of an IPM system for this new and potentially devastating pest in South African vineyards.

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Chapter 5

Conclusions

Trimen's false tiger moth, *Agoma trimenii* (Lepidoptera: Agaristidae), recently developed pest status in vineyards in the Northern Cape and Limpopo (Groblersdal area) provinces of South Africa. As little is known about the biology and behaviour of Trimen's false tiger moth, no official monitoring methods or economic thresholds exist. Consequently, management and control options are lacking. Hence, the overall aim of this study was to generate knowledge on the biology and seasonal occurrence of *A. trimenii* in Southern African vineyards and investigate the potential of various biological control options in an integrated pest management system. The objectives of this study were firstly, to gain greater insight into the biology and seasonal development of *A.trimenii* by testing various monitoring strategies in the field and making observations in the laboratory. Secondly, laboratory bioassays were performed against larval and pupal stages of *A. trimenii* to determine the potential of entomopathogenic nematodes and fungi as biological control agents against these stages of the insect. Thirdly, the potential of commercial biopesticides, Delegate®WG, Steward®150 EC and DiPel®DF to control larvae under laboratory conditions was investigated. Furthermore, semi-field trials were performed to test the potential of DiPel®DF against *A. trimenii* larvae when applied at different water volumes and to assess spray coverage on the vines, as well as the residual activity of DiPel®DF.

The first objective was investigated in the field by means of observational studies and visual scouting, morphological observations on the different life stages, light traps and pheromone and live bait traps. From observational studies and visual scouting in the field, it is apparent that moth outbreaks commence during mid-October and that populations begin to decrease towards April, when larvae begin to pupate and overwinter. It is evident that all life stages of *A. trimenii* can be present simultaneously and that generations overlap on more than one occasion. From observations in the field, it appears that the earliest instars are present on the top leaves and shoots of the vines (± 3 m high). This indicates to growers that control programs must consider the positioning of the early instars, if they are to achieve control, before larval feeding causes extensive foliar damage. If *A. trimenii* is to be targeted for control while in the larval stage, management strategies must be timed correspondingly to the period when neonate

larvae are hatching from eggs. From field observations, it appears that larvae measuring ± 3 cm in length cause the most foliar damage. This highlights the importance of applying control programs before larvae measure ± 3 cm. *A. trimenii* pupae were found in soil mounds around the vines. Different larval stages of *A. trimenii* display various behaviours that may possibly serve as defence mechanisms. These attributes and behaviours, including regurgitation and dropping via silken threads, all suggest that *A. trimenii* may have potential natural predators from their places of origin, but no predation was observed in this study. It is recommended that visual scouting occurs at longer timed intervals over the entire growing season. This can be focused on vineyard hot spots, but it is useful to spread scouting across the vineyard to know where moth activity is greatest.

Of the monitoring methods tested in this study including light traps, pheromone traps and live bait traps, light traps proved the most successful strategy with the added benefit of doubling up as a mass control option. It is useful for growers to test the effectivity of mercury light traps against ultraviolet blue light traps in vineyards for the monitoring and control of *A. trimenii* moths, with the prospect for an *A. trimenii* pheromone lure holding potential as an alternative monitoring strategy. Future research and application of this lure may result in potential monitoring and control options. From field observations, it can be suggested that pheromone traps for the capture of *A. trimenii* be placed in the field during early October, before the first post-winter peak in moth activity. Pheromone trap counts can be used to calculate a degree-day spray timing model. The degree-day calculation indicates to growers when egg hatch will occur and when the next generation should begin to fly.

The quarantine status of *A. trimenii* limited the transportation of larvae and moths to suitable laboratories in the Western Cape. *Agoma trimenii* populations reared in captivity can allow for accurate timing of all life stages, survivorship, developmental times of all life stages and behavioural traits. Outstanding aspects regarding the biology of *A. trimenii*, such as the exact times of overlapping generations, could be resolved by relating observations of *A. trimenii* colonies in captivity, reared on an appropriate diet and environmental conditions, to field conditions. Additionally, rearing a colony could provide information on larval instars which is imperative for mortality-survivorship research based on life tables, as well as population modelling.

The second objective was fulfilled by testing the pathogenicity of local EPN species *Steinernema yirgalemense* and *Heterorhabditis noenieputensis*, and commercially available

EPFs isolates *Beauveria bassiana* and *Metarhizium anisopliae,* to the larval and pupal stages of *A. trimenii.* The pathogenicity of the two local EPN species was screened against larvae and pupae, using a concentration of 100 infective juveniles (IJs) in 50 µl of water. The pathogenicity of the two EPF isolates was screened against larvae and pupae by means of a dipping test undertaken at a concentration of 0.2 ml/500 ml water and 0.5 g/500 ml water, respectively. Good control potential against *A. trimenii* was exhibited by both EPN and EPF isolates, when they were tested against the larval stage of *A. trimenii*. In contrast, the pupal stage showed no sign of infection from either the EPN or the EPF isolates.

Testing EPN and EPF isolates on the prepupal stage of *A. trimenii* holds potential for controlling the population during the time of no damage to crops. Testing all EPN and EPF isolates against the prepupal stage of *A. trimenii* should be the next step in the screening of bioassays. Infection can possibly occur during the short window period of the last larval instar that spends a short amount of time in the soil, prior to pupation and the length of time taken for the newly developed moths to emerge from the soil. As the results were determined under controlled laboratory conditions, whether the EPN and EPF isolates can perform as effectively under semi-field conditions remains to be tested. The EPN and EPF isolates should be tested in additional bioassays, focusing on factors influencing effectivity, and should be evaluated under semi-field and field conditions, so as to enable the investigation of their potential for incorporation into a biological integrated pest management strategy. Additionally, research should be directed at investigating the effect of the EPF isolates, available as commercial products, either individually, or in combination with EPNs as potential biological control agents, against *A. trimenii*. It should also consider the indirect effects of EPNs and EPF on the pupal stage of *A. trimenii*, so as to broaden the existing control options.

The final objective involved the testing of susceptibility of larvae to two commercially available biopesticides, Delegate®WG and DiPel®DF, and a chemical product, Steward®150 EC under laboratory conditions. Semi-field trials were performed to test the potential effectivity of DiPel®DF against larvae, when applied at different water volumes (50g/1000L/ha and 42g/1200L/ha) and to assess spray coverage on top and bottom leaves of vines, as well as the residual activity of DiPel®DF. Both Delegate®WG and Steward®150 EC proved effective against the larval stages of *A. trimenii*, however a trade-off exists between the fast action of Delegate®WG and the longer persistence of activity of Steward®150 EC. Results from laboratory bioassays showed that the recommended dose of DiPel®DF, according to the product

label, is more effective at controlling *A. trimenii* larvae (in comparison to halved and doubled dosages), as 100% mortality was achieved after 5 days of application

Results from semi-field trials suggested that increasing the water volume of a spray application of DiPel®DF will result in greater larval mortality on leaves on the bottom of the vines, as a possible result of a greater spray coverage and subsequent larval mortality, highlighting the importance of achieving maximum spray coverage.

Efforts to improve spray coverage of DiPel®DF, possibly by aerial spraying or adhering to appropriate spraying protocols, are imperative for effective application specifically on top leaves of the vine canopy, as the most damaging stages of *A. trimenii* are located at these sites. It is suggested that knowledge of pest resistance, susceptibility of different life stages to all tested products and the subsequent appropriate timing of spray application, will prove advantageous in developing control programmes. Future research should be directed at investigating the effects of all products against *A. trimenii* under field conditions, either simultaneously or in rotation as part of an integrated pest management system for this new and potentially devastating pest in South African vineyards.